SUPPLEMENTARY INFORMATION

Ribose 2'-*O*-methylation provides a molecular signature for MDA5-dependent distinction of self and non-self mRNA

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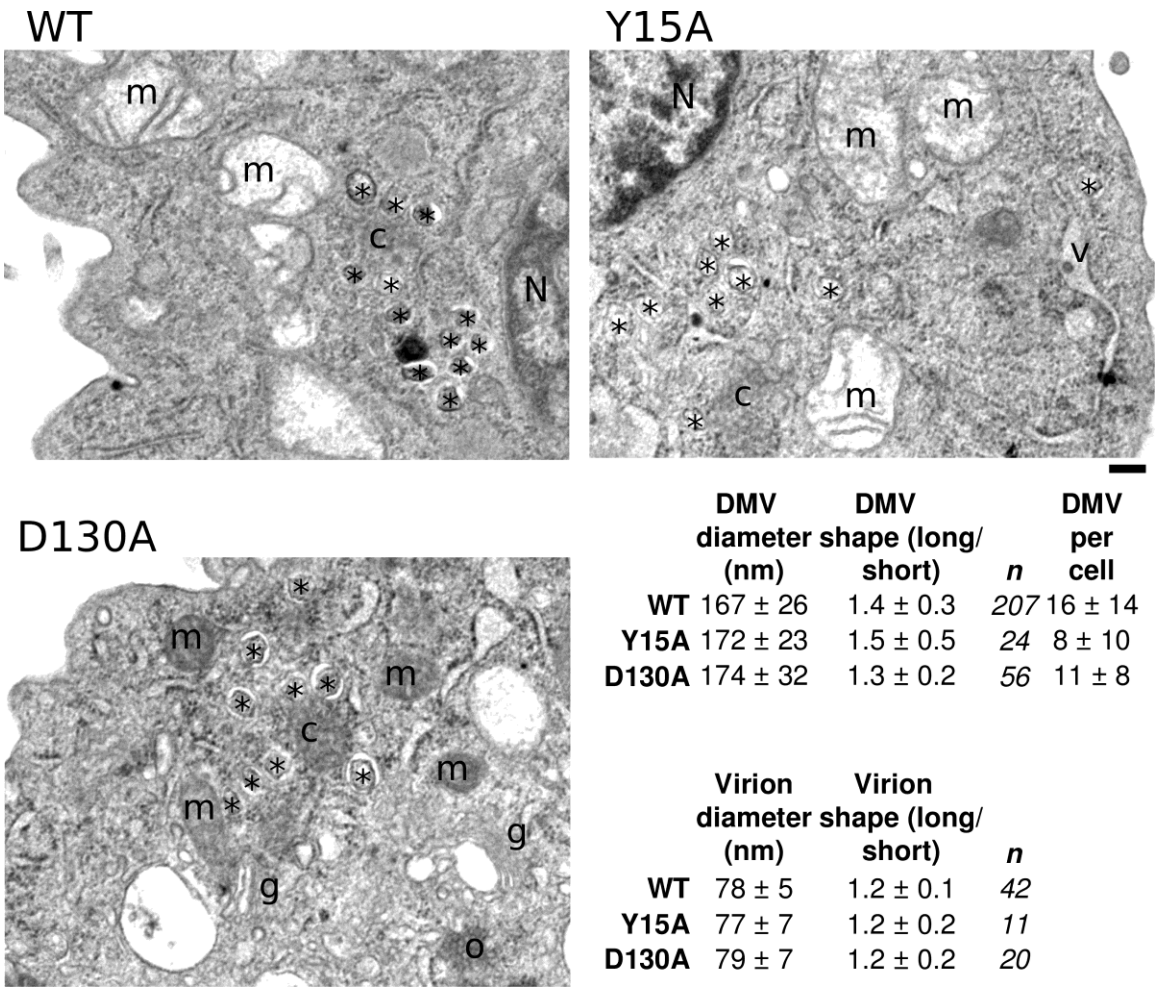
Supplementary Figure 1



	DMV diameter (nm)	DMV shape (long/ short)	*n*	DMV per cell
WT	167 ± 26	1.4 ± 0.3	*207*	16 ± 14
Y15A	172 ± 23	1.5 ± 0.5	*24*	8 ± 10
D130A	174 ± 32	1.3 ± 0.2	*56*	11 ± 8

	Virion diameter (nm)	Virion shape (long/ short)	*n*
WT	78 ± 5	1.2 ± 0.1	*42*
Y15A	77 ± 7	1.2 ± 0.2	*11*
D130A	79 ± 7	1.2 ± 0.2	*20*

Supplementary Figure 1. Morphology, size and number of MHV-induced DMVs are not affected by 2'-O-MTase-deficiency. DMVs were analysed by electron microscopy of ultrathin sections of MHV-A59-, MHV-Y15A-, or MHV-D130A-infected (MOI=1) mouse L929 cells at 5 h p.i.. Virus-induced features including convoluted membranes (c), DMVs (*) and virions (v), as well as cellular features including the nucleus (n), Golgi (g), mitochondria (m) and ordered smooth endoplasmic reticulum (o) are indicated. Quantitative data describing DMV and virions visible in these micrographs are shown at lower right. The average of the longest and shortest visible diameter was taken as a measure of size. The ratio of the longest to the shortest diameter was taken as a measure of shape. The number of DMVs per cell includes only DMV-containing cells. Scale bar denotes 200 nm.

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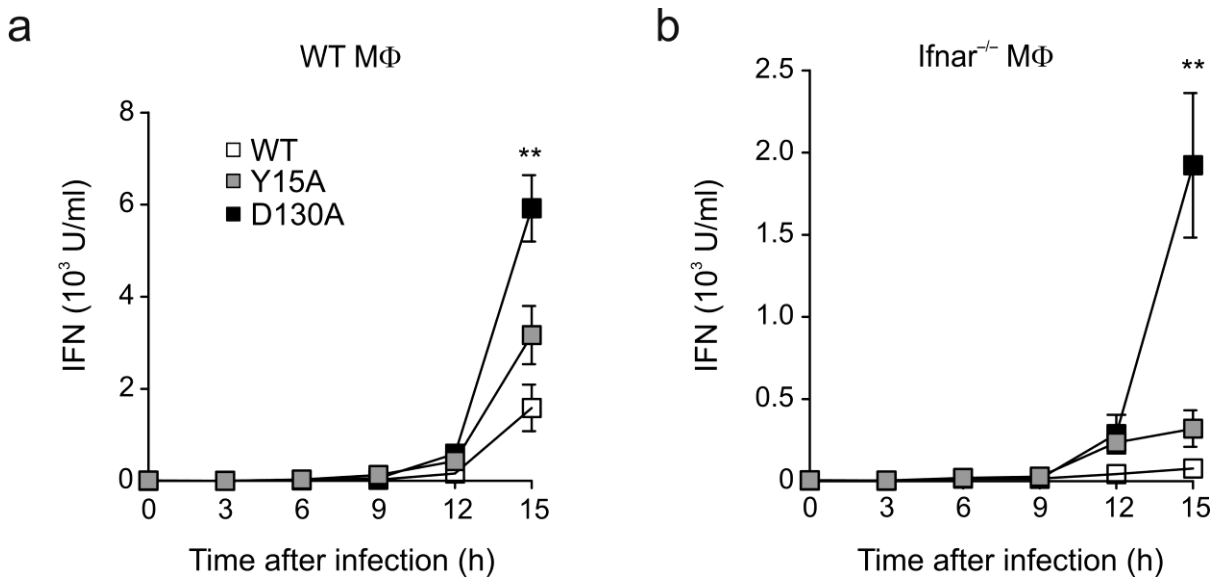
Supplementary Figure 2



Supplementary Figure 2. MHV 2'-O-MTase mutants induce increased IFN-I secretion. a,b) Kinetics of IFN-I secretion induced by MHV 2'-O-MTase mutants was analyzed by bioassay for IFN-I. MΦs (1×10^6) derived from wild-type (**a**), or IFNAR-deficient (**b**) mice were infected (MOI=1) with MHV-A59, MHV-Y15A, or MHV-D130A and IFN-I in cell culture supernatants was determined at indicated time points p.i.. Results represent the mean ±SEM of two independent experiments (n=4). Statistical analysis was performed using unpaired Student's t-test (**, $p < 0.01$).

Supplementary Table 1. Methylation of viral RNA in selected cytoplasmically-replicating virus families infecting multicellular eukaryotes.

Virus class or Order	Family	Genus	RrmJ-like Domain	Function	Reference	mRNA 5' Structure[a]	Reference	Notes
ssRNA(+) Viruses								
Nidovirales	Coronaviridae	All	nsp16	2OMT	47	^{7m}GpppA$^{2'Om}$ or ^{7m}GpppG$^{2'Om}$	48,49	*b*
Nidovirales	Roniviridae	Okavirus	MHV nsp16 homolog	MTh[c]	50	*not determined*		*d*
Nidovirales	Arteriviridae	Arterivirus	*nd*[e]			^{7m}GpppA$^{2'Om}$ or ^{7m}GpppG$^{2'Om}$	51	*f*
Picornavirales	All	All	*nd*			VPg and IRES	52	
Tymovirales	All	All	*nd*			^{7m}GpppG	53	*g*
-	Astroviridae	All	*nd*			*not determined*		*f*
-	Barnaviridae	Barnavirus	*nd*			VPg	54	
-	Bromoviridae	All	*nd*			^{7m}GpppG	55	*g*
-	Caliciviridae	All	*nd*			VPg	56	
-	Closteroviridae	All	*nd*			*not determined*		*g*
-	Flaviviridae	Flavivirus	NS5	N7/2OMT	57	^{7m}GpppA$^{2'Om}$	58	
-	Flaviviridae	Pestivirus	*nd*			IRES	59	
-	Flaviviridae	Hepacivirus	*nd*			IRES	60	
-	Hepeviridae	Hepevirus	*nd*			^{7m}Gppp	61	*h,i*
-	Luteoviridae	All	*nd*			Uncapped	62	
-	Nodaviridae	All	*nd*			^{7m}GpppG	63	
-	Potyviridae	All	*nd*			VPg and IRES	64	
-	Tetraviridae	All	*nd*			*not determined*		*g*
-	Togaviridae	All	nsp2	MTh[j]	65	^{7m}GpppA	66	*k*
-	Tombusviridae	All	*nd*			^{7m}GpppA	67	
-	Virgaviridae	All	*nd*			^{7m}GpppG	68	*g*
ssRNA(-) Viruses[l]								
Mononegavirales	Rhabdoviridae	All	L	N7/2OMT	69	^{7m}GpppA$^{2'Om}$ or ^{7m}GpppN	70	
Mononegavirales	Filoviridae	All	L	MTh[c]	71	^{7m}Gppp	72	*i*
Mononegavirales	Paramyxoviridae	All	L	MTh[c]	73	^{7m}GpppG	74	
-	Arenaviridae	Arenavirus	*nd*			Methylated cap	75	*m*
-	Bunyaviridae	All	*nd*			^{7m}Gppp	76	*i,m*
dsRNA Viruses[n]								
-	Birnaviridae	All	*nd*			VPg	77	
-	Reoviridae	All	λ2/Cap	MTh[c]	78	^{7m}GpppN$^{2'Om}$	79	*o*
dsDNA Viruses[p]								
-	Asfarviridae	Asfivirus	EP424R	MTh[c]	80	^{7m}GpppA$^{2'Om}$	81	
-	Mimiviridae	Mimivirus	L511	MTh[c]	82	*not determined*		
-	Poxviridae	All	VV_vp39 homologs	2OMT	83	^{7m}Gppp^{6m}A$^{2'Om}$	84	*q*
-	Phycodnaviridae	All	*nd*			*not determined*		*r*

[a]Including genomic RNA structure data for positive-sense single-stranded RNA viruses only.
[b]Coronavirus nsp14 is an N7MT.
[c]Indicates detection of one domain with 2OMT homology.
[d]It is unclear whether roniviruses also encode a homolog of the coronavirus nsp14 N7MT [48].
[e]Not detected.
[f]2OMT and N7MT homologs not detected.
[g]One domain has N7MT homology [65,85].
[h]Hepatitis E virus p110 has N7MT activity [86].
[i]Viral RNAs interact with ^{7m}G-cap binding antisera.
[j]A homologous domain is present, but active site residues are not conserved.

[k]One domain of nsp1 has N7MT homology, and internal RNA methylation has been reported [65,66].
[l]Omitting bornavirus and nucleorhabdoviruses, which replicate in the nucleus.
[m]The cap structure of viral mRNA is acquired from host mRNA [87,88].
[n]Omitting the poorly characterized genomes of the Endornaviridae, Partitiviridae and Picobirnaviridae.
[o]Omitting the poorly characterized genomes of Fijivirus Mimoreovirus and Dinovernavirus.
[p]Omitting Iridoviridae which carry out most or all mRNA synthesis in the nucleus.
[q]Conserved poxvirus VV D1-like proteins have N7MT homology [89].
[r]Homologs of chlorella virus A103R have N7MT activity [90]

References for Supplementary Table 1

47 Decroly, E. et al., Coronavirus nonstructural protein 16 is a cap-0 binding enzyme possessing (nucleoside-2'O)-methyltransferase activity. *J Virol* **82** (16), 8071 (2008).

48 This study.

49 van Vliet, A. L., Smits, S. L., Rottier, P. J., and de Groot, R. J., Discontinuous and non-discontinuous subgenomic RNA transcription in a nidovirus. *EMBO J* **21** (23), 6571 (2002); Lai, M. M. and Stohlman, S. A., Comparative analysis of RNA genomes of mouse hepatitis viruses. *J Virol* **38** (2), 661 (1981).

50 Gorbalenya, A. E., Enjuanes, L., Ziebuhr, J., and Snijder, E. J., Nidovirales: evolving the largest RNA virus genome. *Virus Res* **117** (1), 17 (2006).

51 Sagripanti, J. L., Zandomeni, R. O., and Weinmann, R., The cap structure of simian hemorrhagic fever virion RNA. *Virology* **151** (1), 146 (1986).

52 Le Gall, O. et al., Picornavirales, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. *Arch Virol* **153** (4), 715 (2008).

53 Briand, J. P., Keith, G., and Guilley, H., Nucleotide sequence at the 5' extremity of turnip yellow mosaic virus genome RNA. *Proc Natl Acad Sci U S A* **75** (7), 3168 (1978); Klein, C. et al., Physical and functional heterogeneity in TYMV RNA: evidence for the existence of an independent messenger coding for coat protein. *Nucleic Acids Res* **3** (11), 3043 (1976).

54 Revill, P. A., Davidson, A. D., and Wright, P. J., Mushroom bacilliform virus RNA: the initiation of translation at the 5' end of the genome and identification of the VPg. *Virology* **249** (2), 231 (1998).

55 Dasgupta, R., Shih, D. S., Saris, C., and Kaesberg, P., Nucleotide sequence of a viral RNA fragment that binds to eukaryotic ribosomes. *Nature* **256** (5519), 624 (1975); Pinck, L., The 5'-end groups of alfalfa mosaic virus RNAs are m7G5'ppp5'Gp. *FEBS Lett* **59** (1), 24 (1975).

56 Schaffer, F. L., Ehresmann, D. W., Fretz, M. K., and Soergel, M. I., A protein, VPg, covalently linked to 36S calicivirus RNA. *J Gen Virol* **47** (1), 215 (1980).

57 Ray, D. et al., West Nile virus 5'-cap structure is formed by sequential guanine N-7 and ribose 2'-O methylations by nonstructural protein 5. *J Virol* **80** (17), 8362 (2006).

58 Cleaves, G. R. and Dubin, D. T., Methylation status of intracellular dengue type 2 40 S RNA. *Virology* **96** (1), 159 (1979).

59 Poole, T. L. et al., Pestivirus translation initiation occurs by internal ribosome entry. *Virology* **206** (1), 750 (1995).

60 Tsukiyama-Kohara, K., Iizuka, N., Kohara, M., and Nomoto, A., Internal ribosome entry site within hepatitis C virus RNA. *J Virol* **66** (3), 1476 (1992).

61 Kabrane-Lazizi, Y., Meng, X. J., Purcell, R. H., and Emerson, S. U., Evidence that the genomic RNA of hepatitis E virus is capped. *J Virol* **73** (10), 8848 (1999).

62 Allen, E., Wang, S., and Miller, W. A., Barley yellow dwarf virus RNA requires a cap-independent translation sequence because it lacks a 5' cap. *Virology* **253** (2), 139 (1999).

63 Dasgupta, R. et al., Primary and secondary structure of black beetle virus RNA2, the genomic messenger for BBV coat protein precursor. *Nucleic Acids Res* **12** (18), 7215 (1984).

64 Carrington, J. C. and Freed, D. D., Cap-independent enhancement of translation by a plant potyvirus 5' nontranslated region. *J Virol* **64** (4), 1590 (1990).

65 Rozanov, M. N., Koonin, E. V., and Gorbalenya, A. E., Conservation of the putative methyltransferase domain: a hallmark of the 'Sindbis-like' supergroup of positive-strand RNA viruses. *J Gen Virol* **73 (Pt 8)**, 2129 (1992).

66 Dubin, D. T. et al., Sindbis virus messenger RNA: the 5'-termini and methylated residues of 26 and 42 S RNA. *Virology* **77** (2), 457 (1977).

67 Xiong, Z. and Lommel, S. A., The complete nucleotide sequence and genome organization of red clover necrotic mosaic virus RNA-1. *Virology* **171** (2), 543 (1989).

68 Zimmern, D., The 5' end group of tobacco mosaic virus RNA is m7G5' ppp5' Gp. *Nucleic Acids Res* **2** (7), 1189 (1975); Haidar, M. A. and Hirth, L., 5'-terminal structure of tobacco rattle virus RNA: evidence for polarity of reconstitution. *Virology* **76** (1), 173 (1977).

[69] Li, J., Wang, J. T., and Whelan, S. P., A unique strategy for mRNA cap methylation used by vesicular stomatitis virus. *Proc Natl Acad Sci U S A* **103** (22), 8493 (2006).
[70] Abraham, G., Rhodes, D. P., and Banerjee, A. K., The 5' terminal structure of the methylated mRNA synthesized in vitro by vesicular stomatitis virus. *Cell* **5** (1), 51 (1975); Colonno, R. J. and Stone, H. O., Newcastle disease virus mRNA lacks 2'-O-methylated nucleotides. *Nature* **261** (5561), 611 (1976).
[71] Feder, M., Pas, J., Wyrwicz, L. S., and Bujnicki, J. M., Molecular phylogenetics of the RrmJ/fibrillarin superfamily of ribose 2'-O-methyltransferases. *Gene* **302** (1-2), 129 (2003).
[72] Weik, M. et al., Ebola virus VP30-mediated transcription is regulated by RNA secondary structure formation. *J Virol* **76** (17), 8532 (2002).
[73] Bujnicki, J. M. and Rychlewski, L., In silico identification, structure prediction and phylogenetic analysis of the 2'-O-ribose (cap 1) methyltransferase domain in the large structural protein of ssRNA negative-strand viruses. *Protein Eng* **15** (2), 101 (2002).
[74] Barik, S., The structure of the 5' terminal cap of the respiratory syncytial virus mRNA. *J Gen Virol* **74 (Pt 3)**, 485 (1993).
[75] Carter, M. F., Biswal, N., and Rawls, W. E., Characterization of nucleic acid of pichinde virus. *J Virol* **11** (1), 61 (1973).
[76] Hacker, D., Rochat, S., and Kolakofsky, D., Anti-mRNAs in La Crosse bunyavirus-infected cells. *J Virol* **64** (10), 5051 (1990); Vialat, P. and Bouloy, M., Germiston virus transcriptase requires active 40S ribosomal subunits and utilizes capped cellular RNAs. *J Virol* **66** (2), 685 (1992).
[77] Persson, R. H. and Macdonald, R. D., Evidence that infectious pancreatic necrosis virus has a genome-linked protein. *J Virol* **44** (2), 437 (1982).
[78] Mao, Z. X. and Joklik, W. K., Isolation and enzymatic characterization of protein lambda 2, the reovirus guanylyltransferase. *Virology* **185** (1), 377 (1991).
[79] Both, G. W., Furuichi, Y., Muthukrishnan, S., and Shatkin, A. J., Ribosome binding to reovirus mRNA in protein synthesis requires 5' terminal 7-methylguanosine. *Cell* **6** (2), 185 (1975).
[80] Saha, N. and Shuman, S., Effects of alanine cluster mutations in the D12 subunit of vaccinia virus mRNA (guanine-N7) methyltransferase. *Virology* **287** (1), 40 (2001).
[81] Salas, M. L., Kuznar, J., and Vinuela, E., Polyadenylation, methylation, and capping of the RNA synthesized in vitro by African swine fever virus. *Virology* **113** (2), 484 (1981).
[82] Raoult, D. et al., The 1.2-megabase genome sequence of Mimivirus. *Science* **306** (5700), 1344 (2004).
[83] Schnierle, B. S., Gershon, P. D., and Moss, B., Cap-specific mRNA (nucleoside-O2'-)-methyltransferase and poly(A) polymerase stimulatory activities of vaccinia virus are mediated by a single protein. *Proc Natl Acad Sci U S A* **89** (7), 2897 (1992).
[84] Wei, C., Gershowitz, A., and Moss, B., N6, O2'-dimethyladenosine a novel methylated ribonucleoside next to the 5' terminal of animal cell and virus mRNAs. *Nature* **257** (5523), 251 (1975); Wei, C. M. and Moss, B., Methylated nucleotides block 5'-terminus of vaccinia virus messenger RNA. *Proc Natl Acad Sci U S A* **72** (1), 318 (1975).
[85] Gordon, K. H., Johnson, K. N., and Hanzlik, T. N., The larger genomic RNA of Helicoverpa armigera stunt tetravirus encodes the viral RNA polymerase and has a novel 3'-terminal tRNA-like structure. *Virology* **208** (1), 84 (1995); Karasev, A. V. et al., Complete sequence of the citrus tristeza virus RNA genome. *Virology* **208** (2), 511 (1995).
[86] Magden, J. et al., Virus-specific mRNA capping enzyme encoded by hepatitis E virus. *J Virol* **75** (14), 6249 (2001).
[87] Raju, R. et al., Nontemplated bases at the 5' ends of Tacaribe virus mRNAs. *Virology* **174** (1), 53 (1990); Morin, B. et al., The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. *PLoS Pathog* **6** (9).
[88] Reguera, J., Weber, F., and Cusack, S., Bunyaviridae RNA polymerases (L-protein) have an N-terminal, influenza-like endonuclease domain, essential for viral cap-dependent transcription. *PLoS Pathog* **6** (9).
[89] Mao, X. and Shuman, S., Intrinsic RNA (guanine-7) methyltransferase activity of the vaccinia virus capping enzyme D1 subunit is stimulated by the D12 subunit. Identification of amino acid residues in the D1 protein required for subunit association and methyl group transfer. *J Biol Chem* **269** (39), 24472 (1994).
[90] Ho, C. K., Van Etten, J. L., and Shuman, S., Expression and characterization of an RNA capping enzyme encoded by Chlorella virus PBCV-1. *J Virol* **70** (10), 6658 (1996).