Supporting Information

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SI Text

SI Methods. Analysis of Marseillevirus genome. Protein-coding genes were predicted using GeneMark.hmm 2.0 (1) with the following modifications: (*i*) eight short (<50 aa) sequences with no matches in the NCBI Refseq database (2) were eliminated; and (*ii*) intergenic spaces were screened using BLASTX against Refseq database to identify potential missed genes and gene fragments; all ORFs were searched against the Refseq to check gene boundaries; as a result, in 28 cases, ORF boundaries were manually corrected.

The 457 translated protein sequences were searched against the NCBI Refseq and env_nr (environmental nonredundant) protein sequence databases using BLASTP (3). Conserved domains were identified by searching the Conserved Domain Database (CDD version 2.13) (4) using RPS-BLAST. Best Refseq hits and identified conserved domains were used to annotate 219 Marseillevirus proteins.

Marseillevirus protein families (28 clusters of paralogs) were delineated by similarity-based clustering of Marseillevirus protein sequences using BLASTCLUST with subsequent manual curation. The 17 genes unique (amongst NCLDVs) to Mimivirus and Marseillevirus were identified by analysis of phyletic patterns.

Proteins with putative homologs in Refseq were composed of two categories: (*i*) proteins with best Refseq hits e-values ≤ 0.0001 (161 proteins), and (*ii*) proteins with best Refseq hits E-values between 0.0001 and 0.01. All of the proteins in the second category were examined case by case, and 27 of the 64 were included in the set of evolutionarily conserved proteins of Marseillevirus. Thus, 188 proteins were defined as having homologs in Refseq and used for detailed analysis described below.

Acanthamoeba castellanii Neff draft genome was downloaded from the Human Genome Sequencing Center at Baylor College of Medicine FTP site (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/ AcastellaniNeff/). Marseillevirus proteins were searched against the Acanthamoeba castellanii nucleotide sequences using TBLASTN; for significant hits, Acanthamoeba protein fragments were collected directly from the BLAST output. Of the 188 Marseillevirus proteins used for the analysis, 80 had one or more homologs in Acanthamoeba.

Each of the 188 Marseillevirus proteins was aligned (MUS-CLE; ref. 5) with the following sequences: the top Refseq and environmental hits (clustered by BLASTCLUST if numerous); NCLDV othologous proteins (if relevant); and Acanthamoeba hits (if any). Closely related Marseillevirus proteins were included in the same alignments. Alignments were edited as follow: short sequences (mainly environmental sequences) and sequences that did not match most conserved positions of the alignment were removed manually; gapped and less conserved positions were removed either automatically or manually. Maximum-likelihood trees were built from the alignments whenever possible (that is, except for the cases with too few Refseq homologs or too few informative positions). Maximumlikelihood trees were constructed using the TreeFinder program (6), with the estimated site rates heterogeneity and with the WAG (Whelan and Goldman) substitution model (7). The expected-likelihood weights of 1,000 local rearrangements were used as confidence values of TreeFinder tree branches (8).

The phyletic patterns indicating the presence/absence of the respective gene in each species were used for the construction of the neighbor-joining tree shown in Fig. 4 (phylip3.66; ref. 9).

Sequences of the different primers used in RT-PCR analysis. RNA was extracted from purified virions and submitted to RT-PCR analysis using a subset of virus-specific primers: ORF329 was amplified with primers DNA_polT19F (5'-CGAAGCGTACT-CGAAGAACC-3') and DNA_polT19R (5'-AGACGCTTCTC-TGTCGGGTA-3'), ORF157 with primers A32_ATPase_T19F (5'-TCCGCTTCCTATTTTGTGCT-3') and A32_ATPase_T-19R (5'-TAGGATGAGGGGGCACGATAC-3'), ORF270 with primers A18_helicaseF (5'-CAGAACTCCGTGCTCTTTCC-3') and A18_helicaseR (5'-CGCTCTTCCCTCGTACTGAC-3'), ORF342 with primers capsid_T19F (5'-TAACTGCTGCT-TCCGGTTTT-3') and capsid_T19R (5'-ATCTCCGGAACG-ATTCACAG-3'), ORF310 with primers ThioLox_T19F (5'-A-GAACCACAAGCTCCTCGAA-3') and ThioLox_T19R (5'-G-AAATTTGCCCTGCAAGAAA-3'), ORF203 with primers D6R_helic_T19F (5'-GGCAGTGAGGTTTTGCTCTC-3') and D6R_helic_T19R (5'-CAACAGCGGCTTCTTTAGG-3'), ORF424 with primers S/T_kinase_T19F (5'-GCATCCAAAA-ATCTCCTCCA-3') and S/T_kinase_T19R (5'-GCAACCCGC-ATATTTTCTGT-3'), ORF237 with primers A1L-TF_T19F (5'-AACCGCGGTACTGATGAAAG-3') and A1L-TF_T19R (5'-TTTGTGGTCTGTTGGGAGGT-3'), ORF213 with primers TFIIB_T19F (5'-TTCGGTCCCAAAAGAACTTG-3') and TF-IIB_T19R (5'-GTGCAAACGATGATGACACC-3'), ORF166 with primers Histone2A_T19F (5'-CAACATGCTCGTGTTT-TTGG-3') and Histone2A_T19R (5'-TTTTTGTGGGGGAAA-ACAAGC-3'), and ORF414 with primers histoneH2B_T19F (5'-CAGAAGTTCAAAGGCCAAGC-3') and histoneH2B_T-19R (5'-TTCGCACCTTCTTCGAATCT-3'). Amoeba 18S rRNA gene was amplified with forward primer 5'-AACGAA-AGTTAGGGGATCGAA-3' and reverse primer 5'-ACAAG-CTGCTAGGGGAGTCA-3'.

SI Results. Kinetics and quantification of Marseillevirus replication cycle. The main morphological changes along the replication cycle were studied by direct DNA staining with DAPI (10) of A. castellanii infected cells at different time points p.i. or uninfected, as negative controls. The observed changes concerned VF formation and host cell nucleus modifications. Results are shown in Fig. S5. Fluorescence microscopy pictures showed VF brightly stained at 3 h 30 min p.i. that continued to grow until 6 h 30 min p.i. VF formation kinetics was quantified by using the integrated DAPI fluorescence intensity and varied up to 2.5 \times 10⁶ times compared with negative controls (circle curve), whereas there was no significant variation of staining in infected cell nuclei compared to control cells (diamond curve). However, infected cell nuclei underwent a strong but transient morphological modification between 30 min and 2 h 30 min p.i. observed by fluorescence microscopy. Variation of host cell nucleus morphology was quantified using two parameters: the heterogeneity of DAPI staining (black bar histogram) and the ratio between the number of nuclei with a standard ring-like morphology over the total number of nuclei in the microscope field (white bar histogram). For uninfected control cells this ratio was of 60.3%. For these parameters, findings are shown as a percent variation compared with the uninfected cells. As soon as 30 min p.i., only 18% infected cells showed the nucleus ring-like morphology, whereas at 4 h 30 min p.i., 74% of infected cells recovered this morphology. In the meantime, an increase in DAPI staining heterogeneity (up to 172.8% at 30 min and 111.4% at 4 h 30 min versus 100% for the control cells) (black bar histogram) was observed. The measures were limited to 4 h 30 min p.i. due to the difficulty of clearly identifying the nuclei in amoebae harboring very large and bright VF. Thus Marseillevirus replication cycle shares common features with that of Mimivirus as to interaction with cell nuclei, formation, and growth of an independent VF, but has also specificities such as VF morphology, growth kinetics, synthesis, and assembly of progeny virions.

Detection of viral RNA within Marseillevirus particles. Cellular DNA and RNA contamination was excluded because no 18S amplification product could be detected after PCR and RT-PCR on RNA isolated from purified Marseillevirus. RNA samples were free of Marseillevirus DNA contamination because no amplified product was observed after RT-PCR using Taq polymerase instead of the RT/Taq enzyme mix. Transcripts of genes coding respectively for DNA polymerase (ORF329), capsid protein (ORF342), TFII-like transcription factor (ORF213), and D6R helicase (ORF203) have been detected with specific amplification of cDNA obtained after reverse transcription of viral RNA, suggesting that viral transcripts might be packaged in Marseillevirus particles.

- Lukashin AV, Borodovsky M (1998) GeneMark.hmm: New solutions for gene finding. Nucleic Acids Res 26:1107–1115.
- Pruitt KD, Tatusova T, Klimke W, Maglott DR (2009) NCBI Reference Sequences: Current status, policy and new initiatives. *Nucleic Acids Res* 37:D32–D36.
- Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402.
- Marchler-Bauer A, et al. (2009) CDD: Specific functional annotation with the Conserved Domain Database. Nucleic Acids Res 37:D205–D210.
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.
- Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: A powerful graphical analysis environment for molecular phylogenetics. BMC Evol Biol 4:18.

Coinfection of A. castellanii with purified Marseillevirus, Legionella drancourtii, and Parachlamydia strain BN9. A. castellanii were infected with a mixture of Marseillevirus, Legionella drancourtii, and Parachlamydia strain BN9. At 16 and 24 h p.i., amoebal cells were spotted on microscopes slides using a Cytospin. Then, nucleic acids were stained using DAPI (Molecular Probes) or Hemacolor (Merck), according to the manufacturer's instructions. The morphology of each DAPI or Hemacolor-stained microorganism inside the amoebal cells was identified by comparison with amoeba infections with each microorganism alone. As shown in Fig. 5, VF of Marseillevirus, strongly stained with DAPI (Fig. 5A Left) or with Hemacolor (purple color) (Fig. 5A Right), can coexist in the same amoebal cytoplasm with both Legionella drancourtii (long rod-shaped bacteria, blue colored in Hemacolor staining) and Parachlamydia (coccoid-shaped bacteria, blue-colored in hemacolor staining). Thus, coinfected amoebal cells could be the host for simultaneous bacteria and virus multiplication.

- Whelan S, Goldman N (2001) A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. *Mol Biol Evol* 18:691–699.
- Strimmer K, Rambaut A (2002) Inferring confidence sets of possibly misspecified gene trees. Proc Biol Sci 269:137–142.
- Felsenstein J (2005) PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. (Department of Genome Sciences, University of Washington, Seattle).
- Suzan-Monti M, La Scola B, Barrassi L, Espinosa L, Raoult D (2007) Ultrastructural characterization of the giant volcano-like virus factory of Acanthamoeba polyphaga Mimivirus. PLoS One 2:e328.



Fig. S1. (a and b) 2D reference gels of the protein extract of Marseillevirus. Proteins were resolved in the first dimension over a pl gradients of 3–10 (a) or 6–11 (b), followed by 10% linear SDS/PAGE for the second-dimension separation. The 2D gels were silver stained. Identified proteins, achieved by MALDI-TOF analysis, are indicated by arrows and listed in .pdf;2009_PDF;/Border [0 0 0]Table S1. Standard molecular weight and p/ range are indicated. HP corresponds to ORF product not detected during ORF prediction. Actin spots correspond to actin proteins from host cells. (*c*–*h*) Posttranslational analysis of Marseillevirus proteins resolved on 2D gel. Viral proteins resolved by 2D gels were revealed by silver staining (c). Glycoproteins were stained with the Pro-Q Emeral 300 glycoprotein kit according to the manufacturer's protocol (d). Western blot analysis using the mouse polyclonal antiserum (1/2,500) prepared against purified viral particles is shown in (e). Phosphorylated proteins were revealed with anti-phosphoserine (1/10,000) (f), anti-phosphothreonine (1/10,000) (g), or anti-phosphotyrosine (1/10,000) (h) mAbs. Standard molecular weight markers are indicated on the left.



Fig. 52. Phylogenetic analysis of conserved NCLDV proteins: (a) D5-like helicase; (b) DNA polymerase; (c) A32-like ATPase; (d) major capsid protein; (e) VLTF2-like transcription factor; (f) A18-like helicase. Maximum-likelihood (ML) trees were constructed using the TreeFinder program, with the estimated site rates heterogeneity and with the WAG substitution model. The expected-likelihood weights of 1,000 local rearrangements were used as confidence values of TreeFinder tree branches. Reference strains used: b1_Helvi, *Heliothis virescens* ascovirus 3e; b1_Spofr, *Spodoptera frugiperda* ascovirus 1a; b1_Trini, *Trichoplusia ni* ascovirus 2c; c1_Afrsw, African swine fever virus; l1_Invir, Invertebrate iridescent virus 3; l2_Invir, Invertebrate iridescent virus 6; l3_Lymdi, Lymphocystis disease virus - isolate China; l5_Ambti, *Ambystoma tigrinum* virus; l5_Frovi, Frog virus 3; l5_Singr, Singapore grouper iridovirus; n2_Acapo, *Acanthamoeba polyphaga* mimivirus; q1_Acatu, *Acanthocystis turfacea Chlorella* virus; q6_Ostvi, *Ostreococcus* virus OsV5; u1_Bovpa, Bovine papular stomatitis virus; u1_Crovi, Croocillepox virus; u1_Ectvi, Ectromelia virus; u1_Fowvi, Fowlpox virus; u1_Goavi, Goatpox virus Pellor; u1_Molco, Molluscum contagiosum virus; u2_Amsmo, *Amsacta moorei* entomopoxvirus; u2_Melsa, *Melanoplus sanguinipes* entomopoxvirus; u3_Mulde, Mule deer poxvirus. Environmental sequences are named by their GenBank accession numbers.



Fig. S2. Continued.

(b)

DN AS

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DNAS

DNA S





Fig. S2. Continued.

DN A C



Fig. S2. Continued.

(f)

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(a) (b)	orf68 :: ::::::::::::::::::::::::::::::::::::	NE FRAS BALL WHEKTP OF 68 : SSEARARS STORE HENDER WEYE WEYETLE OF 68 : SSEARARS STORE HENDER WEYE WEYETLE OF 68 : SOURCE AND STORE HENDER WEYE WEYE WEYETLE OF 68 : SOURCE AND STORE HENDER WEYE WEYETLE OF 68 : SOURCE AND STORE HENDER WEYETLE OF 68 : SOURCE	S UTANSER DESEME FRAST HALL CATERN'T P STUTNER DESEME TARES ALL CATERN'T P STUTNER DELEMENTE & LAU STOTLP STUTNER DELEMENTE & LAU STOTLP STUTNER DELEMENTE & LAU STOTLP STUTNER DELEMENTE & LAU STOTLP - RESEARCH STOTLE ALL STOTLE - RESEARCH STOTLE ALL STOTLE STUTNER DESEMENT - TERMENTE STUTNER DELEMENTE DELEMENTE - TOTLE DEL
(c)	101371 - Huminian Hind Hind 101371 - Huminian Hind Hind 15800862 : FVTSELERIALQ-FG	MINISHIG THANK STORY WEARSERGMTHAYDHIK STORY THATACTECHROKSASE 15800862 : FVTSBLERIALQ-FK HUPVLPLCRKH-HRELHTDT 89108402 : MPADDHS TGHS-Q MUTLACEDBLARKGN 15608288 : AHBER TFA-T NETOGCGHHQLATTGG 15608840 : GYLTBR TFFA-Q H D NETOGCGHHQLATTGG 15608840 : GYLTBR TFFA-Q H D NETOGCGHHQLATTGG 15608840 : GYLTBR TFFA-Q H NPT TGKHL FFI TVADKFILEI KNPE TOKHLEFD TVS-KTITEL KNPE TOKHLEFD FVS-KTITEL	H BIOGROMIC I I BRASSIGNTATOHIK GRAN I BRASSIGNTATOHIK GGREETINTLCTECHRKSASE GGREETINTLCTECHRKSASE GGRETINTLICKH-HRLHTDT THRTINTLICKH-HRLHTDT H D DGRQHFEQVSGWILLEEIQRRDKEK DGRQHFEQVSNWRTPEETQERDKLKE DGRQHYEQVSNWRTPEETQKSDRKE DGRQHYEQVSNWRTPEEQQSSDRKE DGRQHYEQVSNWRSPELIQKNDRKE DGRQHYEQVSNWRSPELIQKNDRKE DGRQHYEQVSNWRSPELTQKSDRKE
	orf156 : SVAEERGYEH orf245 : ELAEDHGFKT orf349 : ECAIKNGYSV orf409 : ECAMKNGYSV orf40 : HCAVKNGYSV orf327 : EQANKNGYST	LRLIQEDVWLERTEWRKEIQHFU LRLIQEDVWDSVDWKSQIKEFI LRILQEDVWDDRTDWKKLLLEHI LRILQEDVWEDRIDWKKLLLEHI LRVLQEDVWSEKIDWRALLLEHV LRILQEDVWRDRVDWKSFLLENI	LGK KEIF KDYETPVVKNLWDETIPESQE KNYEKPTTRRLWED KDYGEPIVKNIAK KDYESPITKNLWETSP
(d)	orf371 : LEKQORAAF orf249 : VARKILKSE orf428 : SRNKRSKSR orf301 : VRKKAMKSSY	RYKTYTEPSGROMDYOGYEHFAI KKEFIMPSGSPFICOGFEPLAL KFKPFLLPSGKTVWIOGFEDIYL GFRDYIFPSGKKIRIOGYENFAI D EK	DLLLKRGTPEENIHNPLKKGIRIPYT QLLLDEGIDEQDILSPSBQCIKIPYE EELLKE-FREEEILVCEQTPYFVPYK DFLLSEGIREDDIFGCYEKGISFPYS
	orf371 : EKEKNRLYNE orf249 : EOSKSHMYHE orf428 : WYSKTEKYLE orf301 : ENSKNSFYFE	DIFVDSINLIVEVKSSWTYKG- DIFVPSINLIIEVKSDWTFGSCG DFFIPSENLFIEVKSDWTFFK- DIFVESRNLVEVKSTWTLREV-	Kneykdlcifklsac reogyktli Gkkkddad Rtikklaac reogyntri Rkgoekktrakleacnalgydtri Ronded Rtikkleac Rsagyktri
	orf249 : VVYRSRSSKKV orf301 : LVIGPRGE	HABINSLDAFLESE- -HASFHBRISPINF /SHFEEKBGIFSQD- HLEDREQ	

Fig. S3. (a) Alignment of the F-box from nine F-box-containing proteins found in Marseillevirus. (b) Five Marseillevirus HNH endonuclease domains aligned with GenBank representatives: 6137348, pdb 1BXLB, Escherichia coli; 137691, Enterobacteria phage lambda; 15150712, Pylaiella littoralis; 29366715, Pseudomonas phage gh-1; 9627446, Enterobacteria phage T7; 30387469, Yersinia pestis phage phiA1122; 3769356, Helicobacter pylori; 16763791, Salmonella typhimurium LT2; 945381, Lactobacillus phage LL-H; 15800862, Escherichia coli O157:H7; 89108402, Escherichia coli W3110; 15608288, Mycobacterium tuberculosis H37Rv; 15608840, Mycobacterium tuberculosis H37Rv. (c and d) Alignment of restriction endonuclease-like proteins found in Marseillevirus. (c) Cluster 4 (eight proteins). (d) Cluster 10 (four proteins). The positions conserved in cd01038 (c) and in cd00523 (d) are indicated in the row above the alignment. The alignment was done by MUSCLE and edited manually.





Fig. S4.1. AP (apurinic) endonuclease family 2 (Marseillevirus orf293)





Fig. 54. (see Fig. S4.1–S4.89). Phylogenetic trees were constructed for 22 Marseillevirus proteins involved in DNA replication, recombination, and repair (Fig. S4.1–S4.22); 9 in nucleotide metabolism (Fig. S4.23–S4.31); 12 in signaling (Fig. S4.32–S4.43); 10 in protein and lipid synthesis, modification, and degradation (Fig. S4.44–S4.53); 16 in transcription and chromatin modification (Fig. S4.54–S4.69); 5 in translation (Fig. S4.70 to S4.74); 8 in miscellaneous functions (Fig. S4.75–S4.82); and 7 of unknown function (Fig. S4.73–S4.89). A color code was used to represent taxonomic groups, *Bacteria* in green, *Archaea* in pink, *Acanthamoeba* in purple, *Dictyostelium* in green-blue, *Entamoeba* in light blue, other eukaryotes in dark blue, Mimiviruses in orange, Marseillevirus in red, other NCLDVs in brown, other viruses and phages in dark, and environmental sequences in yellow. Maximum-likelihood trees were constructed using the TreeFinder program, with the estimated site rates heterogeneity and with the WAG (Whelan and Goldman) substitution model. The expected weights of 1,000 local rearrangements were used as confidence values of TreeFinder tree branches.





Fig. S4.5. HNH family endonuclease (Marseillevirus ORF318)



Fig. S4.6. HNH family endonuclease (Marseillevirus ORF295)



Fig. S4.8. Proliferating Cell Nuclear Antigen (PCNA) (Marseillevirus ORF212)

Fig. S4.7. HNH family endonuclease (Marseillevirus ORF387)



<u>**Fig. S4.9.**</u> putative nuclease linked to bacterial restriction-modification systems (Marseillevirus ORF181)







Fig. S4.10. RuvC-like Holiday Juncition Resolvase (Marseillevirus ORF216)



Fig. S4.12. AlkB, Alkylated DNA repair protein unique in NCLDV (Marseillevirus ORF258)



Fig. S4. Continued.

Fig. S4.16. D5 family helicase-primase (Marseillevirus ORF152)

u2 Amsmo9964401 u2 Melsa9631496

Fig. S4.15. flap-specific endonuclease (poxvirus G5 homolog) (Marseillevirus ORF365)













Fig. S4.20. DNA polymerase elongation subunit (family B) (Marseillevirus ORF329)

Fig. S4.19. ATP-dependent DNA ligase (Marseillevirus ORF72)













Fig. S4.22. helicase superfamily I member (RecD, ATP-dependent exoDNAse (exonuclease V), alpha subunit) (Marseillevirus ORF408)



Fig. S4.24. Dam-like adenine-specific DNA methylase (Marseillevirus ORF17)



Fig. S4.25. bifunctional dihydrofolate reductase-thymidylate synthase (Marseillevirus ORF429)



Fig. S4.27. helicase origin-binding-protein (UL9) like (Marseillevirus ORF211)



Fig. S4.26. Pox A32 (Marseillevirus ORF157)



Fig. S4.28. Ribonucleotide reductase, small chain (Marseillevirus ORF268)









Fig. S4.30. DHH family phosphohydrolase (Marseillevirus ORF131)



Fig. S4.31. P-loop ATPase or GTPase (Marseillevirus ORF326)

Fig. S4.32. Tandem kinase unique to Marseillevirus and Mamavirus (Marseillevirus ORF122)





DNAS



Fig. S4.33. Marseillevirus ORF160

Fig. S4.34. Marseillevirus ORF195











Fig. S4. Continued.

DN A C



DNAS









Fig. S4.42. Marseillevirus ORF427



Fig. S4.44. chaperone protein DnaJ (Marseillevirus ORF269)





Fig. S4.45. Erv1/Alr family protein (poxvirus E10 ortholog) (Marseillevirus ORF310)



Fig. S4.47. Lipase class 3 (Marseillevirus ORF189)

Fig. S4.46. papain-like cysteine peptidase (Marseillevirus ORF285)



Fig. S4.48. metallopeptidase WLM (Marseillevirus ORF278)







By Lenar14919763

Bp Shiso74314442

Ec Plakn221057532

q2 Emihu73852623

10000

7867

6442

9956

Bp Alcbo110834788

Bn Alcsn223480890

760

671

0.2

385

628





Fig. S4.51. peptidoglycan peptidase (Marseillevirus ORF416)



Fig. S4.52. ankyrin repeat protein (Marseillevirus ORF331)













Fig. S4.54. D6/D11-like helicase (Marseillevirus ORF203)



0.2

Fig. S4.56. Histone 2A-domain-containing protein (Marseillevirus ORF166)



DN A C



Fig. S4.57. Histone H3 (Marseillevirus ORF413)



Fig. S4.58. RNA ligase (Marseillevirus ORF197)



















Fig. S4.63. Transcription factor S-II (TFIIS)-domaincontaining protein, RNAP subunit (Marseillevirus ORF222)

<u>Fig. S4.64.</u> RNAP subunit Rpb5 (Marseillevirus ORF383)





Fig. S4.66. Zn-ribbon-like motif (Marseillevirus ORF81)



Fig. S4.67. DNA-directed RNA polymerase subunit alpha; RNA polymerase Rpb1, domains 3-7 (Marseillevirus ORF55)

u2 Amsmo9964535

Fig. S4.68. DNA-directed RNA polymerase subunit alpha; RNA polymerase Rpb1, domains 1-2 (Marseillevirus ORF52)

Fig. S4. Continued.

E9 Vitvi225459758

H 0.2 orf55a Acanthamoeb





Fig. S4.69. DNA-directed RNA polymerase subunit beta (Marseillevirus ORF23)



<u>Fig. S4.71.</u> Translation initiation factor SUI1 (Marseillevirus ORF79)

Fig. S4.70. Translation elongation factor EF-1alpha (Marseillevirus ORF163)



Fig. S4.72. Eukaryotic peptide chain release (translation

termination) factor 1 (Marseillevirus ORF130)

Fig. S4. Continued.





Fig. S4.73. cell division cycle 123 homolog (Marseillevirus ORF400)



Fig. S4.74. eukaryotic translation initiation factor 5 (Marseillevirus ORF304)





Fig. S4.76. mannosyltransferase OCH1-like protein (Marseillevirus ORF162)





Fig. S4.77. calcineurin-like phosphoesterase (Marseillevirus ORF363)









Fig. S4.80. WD repeat protein (Marseillevirus ORF426)





Fig. S4.83. uncharacterized protein conserved in several Fig. S4.84. contains an uncharacterized conserved bacteria and eukaryotes, and mimivirus (low similarity) (Marseillevirus ORF126)

domain found in diverse bacterial and phage proteins (Marseillevirus ORF183)

Fig. S4. Continued.



Fig. S4.85. Uncharacterized protein conserved in bacteria and mimivirus (Marseillevirus ORF104)



Fig. S4.86. uncharacterized protein with homologs in some bacteria (Marseillevirus ORF111)



MIMI_L371 (Marseillevirus ORF230)

Fig. S4.87. uncharacterized, conserved domain in Fig. S4.88. uncharacterized TIM-barrel protein conserved in numerous bacteria (Marseillevirus ORF255)

Fig. S4. Continued.

0.2





Fig. S4. Continued.

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Fig. S5. Kinetics and quantification of Marseillevirus virus factory formation. Uninfected (negative control) or Marseillevirus infected *A. castellanii* at different times postinfection were stained with DAPI. Immunofluorescence pictures were taken using a $100 \times$ magnification lens. Evolution of virus factory (diamonds) was compared with cell nuclei (circles) mean DAPI intensity staining. Transient modification of cell nuclei morphology was expressed as the ratio variation of ring pattern nuclei/modified nuclei normalized to the negative control value (100% value) (white bars). Heterogeneity of cell nuclei DAPI staining was expressed as the standard deviation normalized to the negative control (100% value) (black bars).

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)
Table S4 (PDF)