Supplementary material

Comparative Analysis of the Circular and Highly Asymmetrical *Marseilleviridae* Genomes

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Table S1

Table S1. Complete marseilleviruses sequenced genomes

Strain	Clade	Genome length (nt)	# ORFs	Source	Reference	GenBank Accession
Marseillevirus	A	368454	509	Water of a cooling tower	(Boyer et al., 2009) http://dx.doi.org/10.1073/pnas.0911354106	GU071086
Lausannevirus	B	346754	461	Drinking water treatment plant	(Thomas et al., 2011) http://dx.doi.org/10.1111/j.1462-2920.2011.02446.x	HQ113105
Cannes 8 virus	A	374041	510	Water from a cooling tower	(Aherfi et al., 2013) http://dx.doi.org/10.1007/s11262-013-0965-4	KF261120
Insectomime virus	C	386631	731	Internal organs of Eristalis tenax larvae	(Boughalmi et al., 2013) http://dx.doi.org/10.1159/000354560	HG428764
Tunisvirus	C	380011	540	Water from a decorative fountain	(Aherfi et al., 2014) http://dx.doi.org/10.1007/s00705-014-2023-5	KF483846
Brazilian marseillevirus	D	362276	487	Water sample of an urban lake	(Dornas et al., 2016) http://dx.doi.org/10.3390/v8030076	KT752522
Melbournevirus	A	369360	505	Muddy fresh water	(Doutre et al., 2014) http://dx.doi.org/10.1128/JVI.02414-14	KM275475
Port-miou virus	B	349275	468	Brackish Submarine Spring	(Doutre et al., 2015, p.) http://dx.doi.org/10.1128/genomeA.01148-15	KT428292
Tokyovirus	A	362593	491	Water/soil sample from the Arakawa River	(Takemura, 2016) https://dx.doi.org/10.1264%2Fjsme2.ME16107	Reassembled (Dataset S1)
Noumeavirus	B	376207	507	Muddy sample of fresh water	(Fabre et al., 2017) http://dx.doi.org/10.1038/ncomms15087	KX066233
Golden marseillevirus	E	360610	543	Golden mussels	(Dos Santos et al., 2016) https://doi-org.insb.bib.cnrs.fr/10.1038/srep35237	KT835053
Kurlavirus	B	361368	495	Sewage water sample	(Chatterjee and Kondabagil, 2017) http://dx.doi.org/10.1007/s00705-017-3469-z	KY073338
Marseillevirus shanghai	A	368078	505	Unknown	Unpublished	MG827395

Figure S1

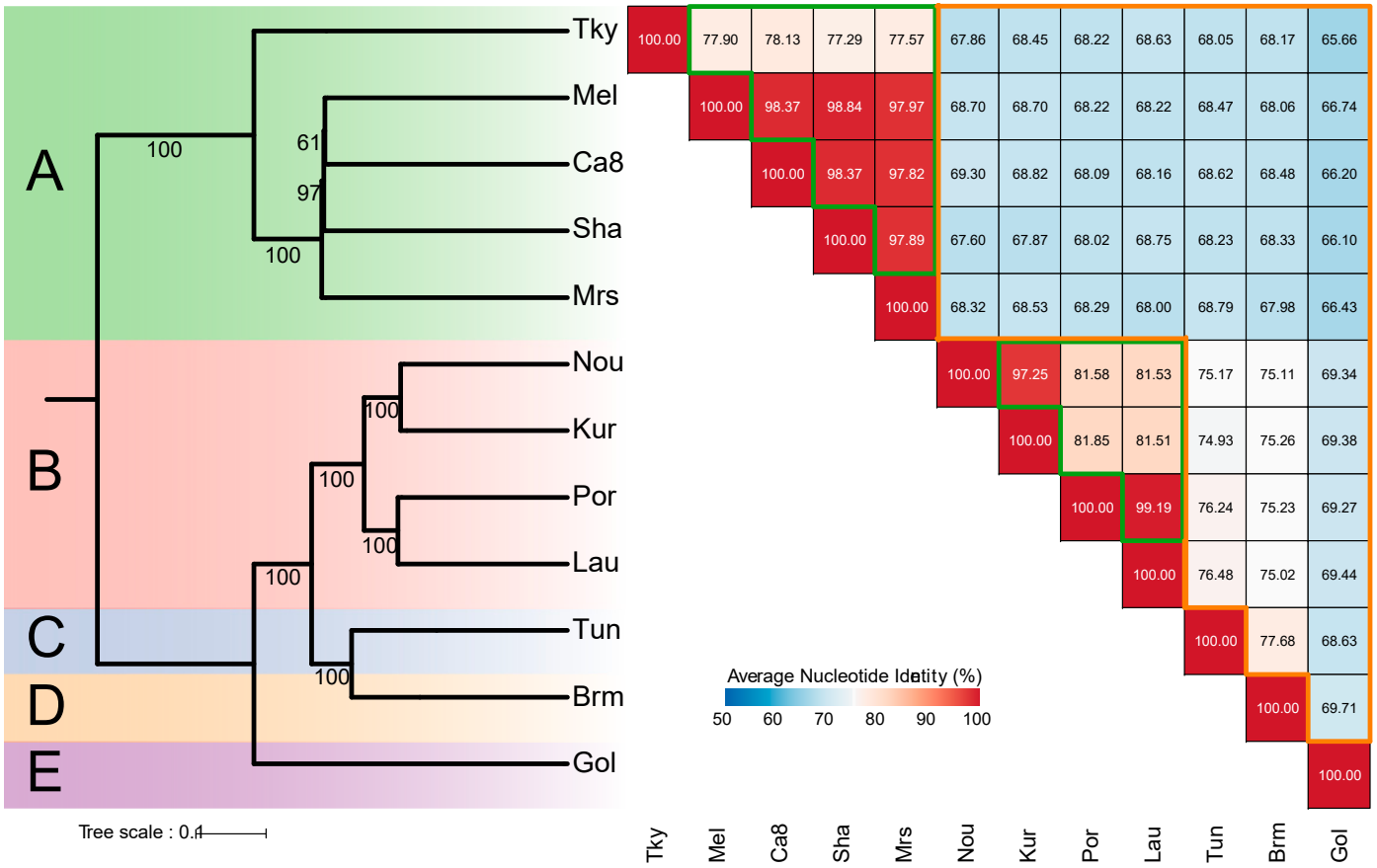

Figure S1. Nucleotide-level genomic similarity between *Marseilleviridae*. The matrix at the right shows the color-coded values of Average Nucleotide Identity (ANI) between pairs of marseilleviruses genomes. Values highlighted in green correspond to intra-clades comparisons while those highlighted in orange correspond to inter-clades comparisons. The phylogenetic tree on the left was computed from the concatenated alignment of single-copy orthologous core genes (see Materials and Methods). Each *Marseilleviridae* clade is labeled and color-coded with tokyovirus (Tky), melbournevirus (Mel), cannes 8 virus (Ca8), marseillevirus shangai (Sha) and marseillevirus (Mrs) in clade A (green); noumeavirus (Nou), kurlavirus (Kur), Port-miou virus (Por) and lausannevirus (Lau) in clade B (red); tunisvirus (Tun) in clade C (blue); Brazilian marseillevirus (Brm) in clade D (orange) and golden marseillevirus (Gol) in clade E (purple).

Figure S2

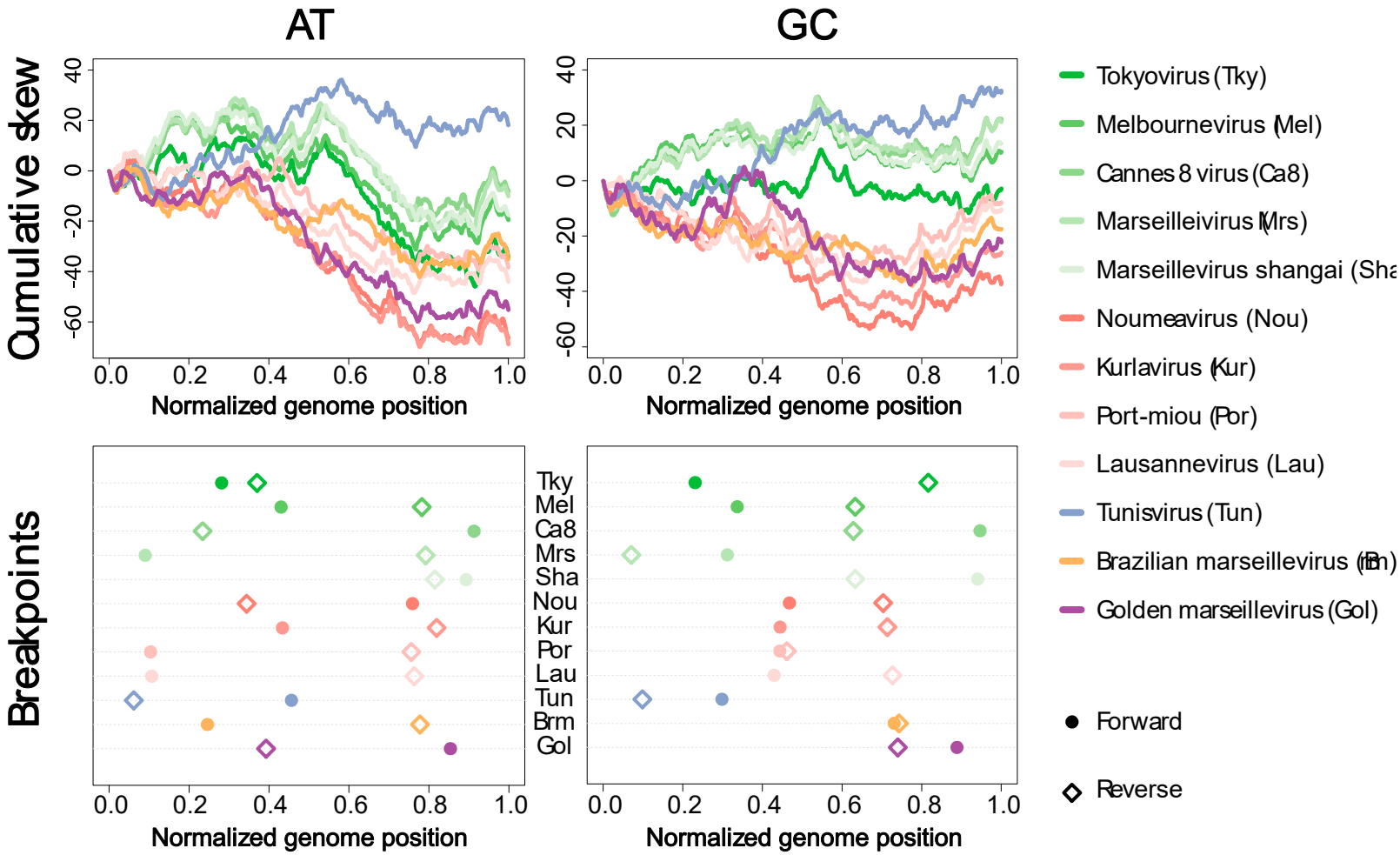

Figure S2. Identification of potential origins of replication using cumulative AT-skew and GC-skew. Each viral genome is color-coded using a similar color for viruses that belong to the same clade (A in green, B in red, C in blue, D in orange and E in purple). Breakpoints in the correlation of cumulative CDS skew and composition skews were calculated using the "rearranged.oriloc" function from the SeqinR R package (see Materials and Methods for details). Genomic positions were normalized by the length of each genome.

Figure S3

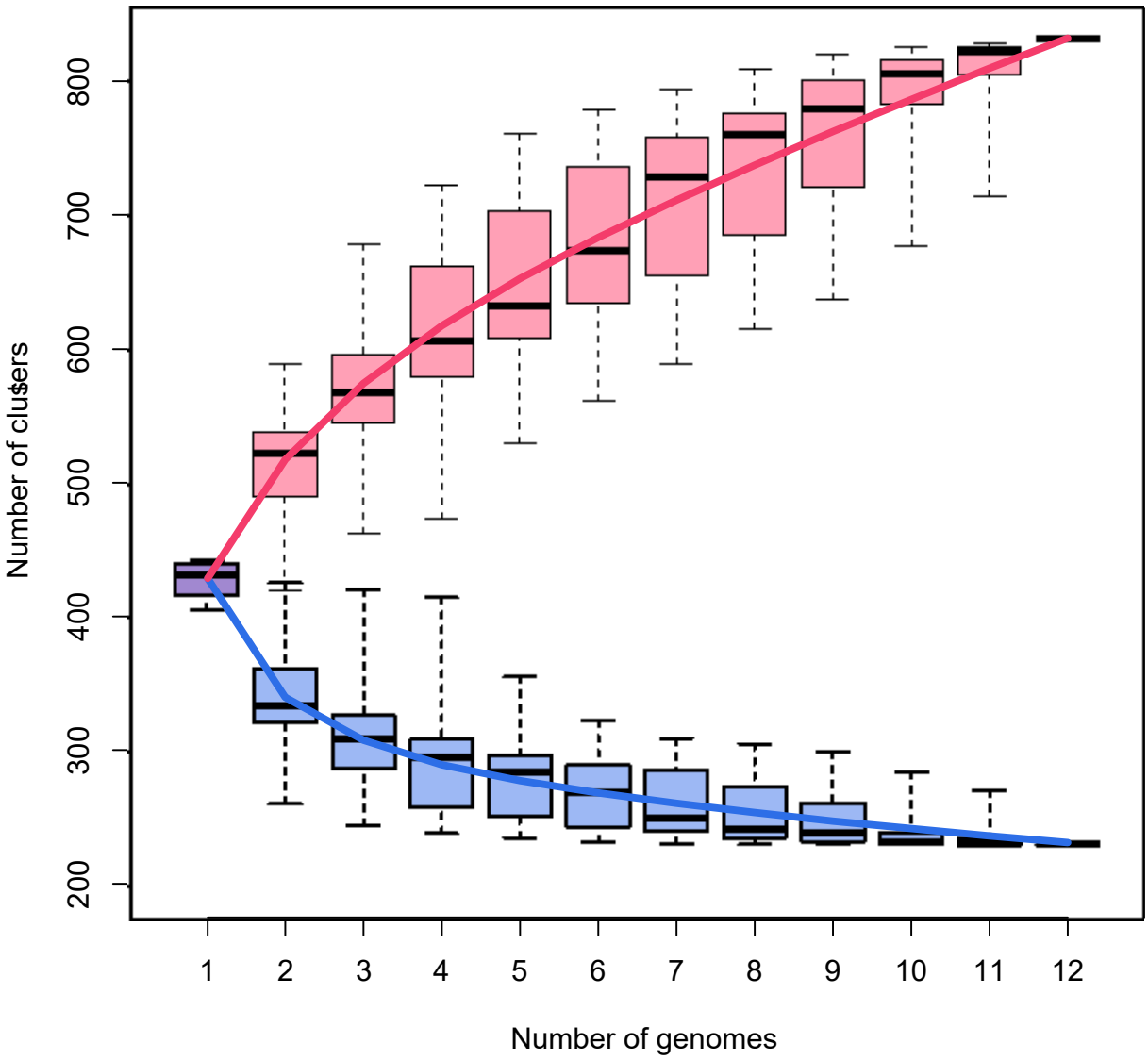

Figure S3. Core-genome and pan-genome of the *Marseilleviridae*. The box plots show the number of shared orthogroups (in blue) and the total number of orthogroups (in pink) as a function of the number of genomes being compared. Box plots show the median, the 25th and 75th percentiles. The whiskers correspond to the extreme data points. The curves correspond to average values.

Figure S4

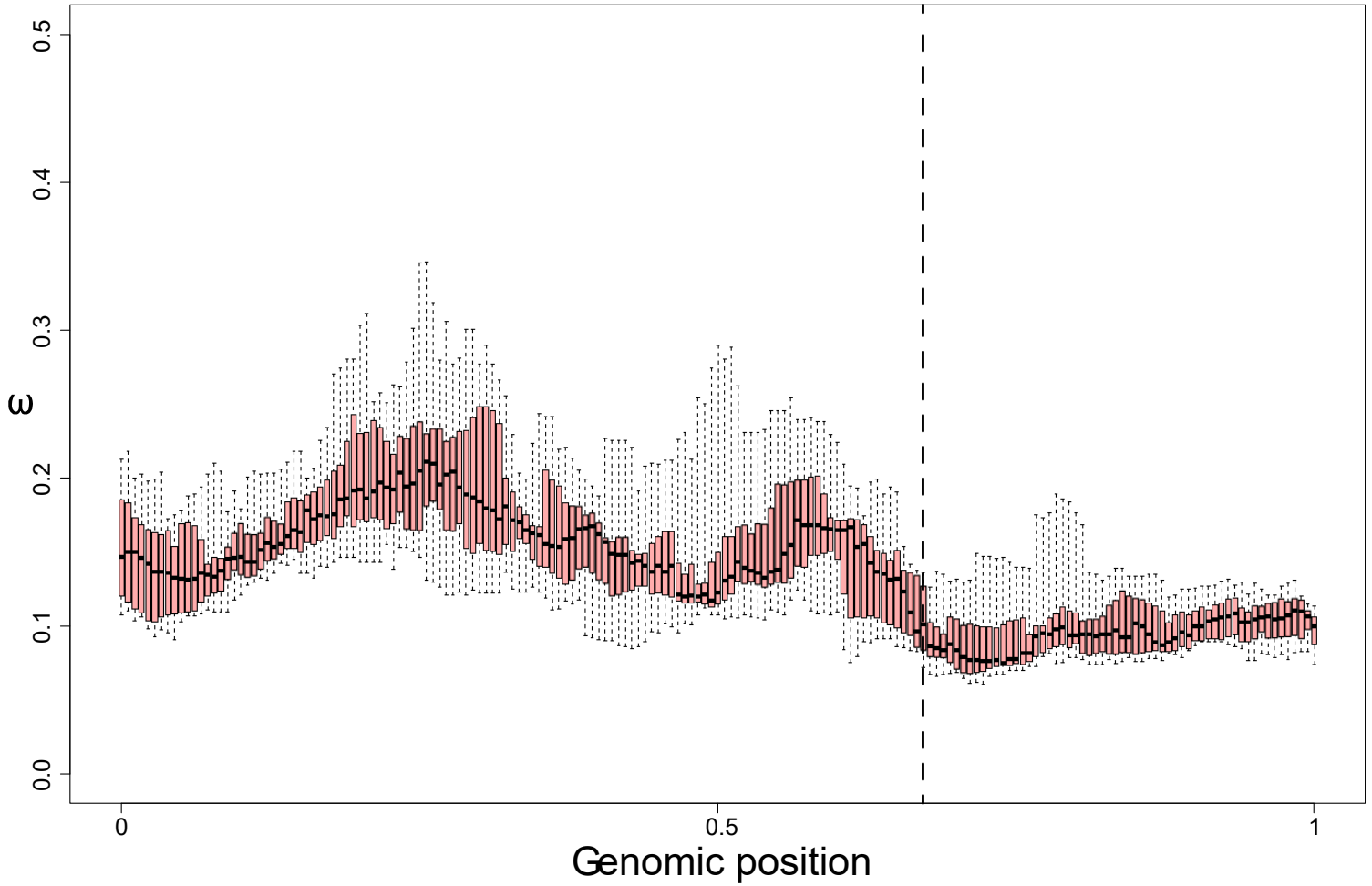

Figure S4. Selection pressure along the *Marseilleviridae* genomes. Omega values were calculated along the marseilleviruses genomes whose genomic positions were normalized by the length of each genome. Omega values were binned along the genome according to the relative genomic positions. Box plots show the median, the 25th and 75th percentiles. The whiskers correspond to the extreme data points. The dashed line separates the core region from the rest of the genome.

Figure S5

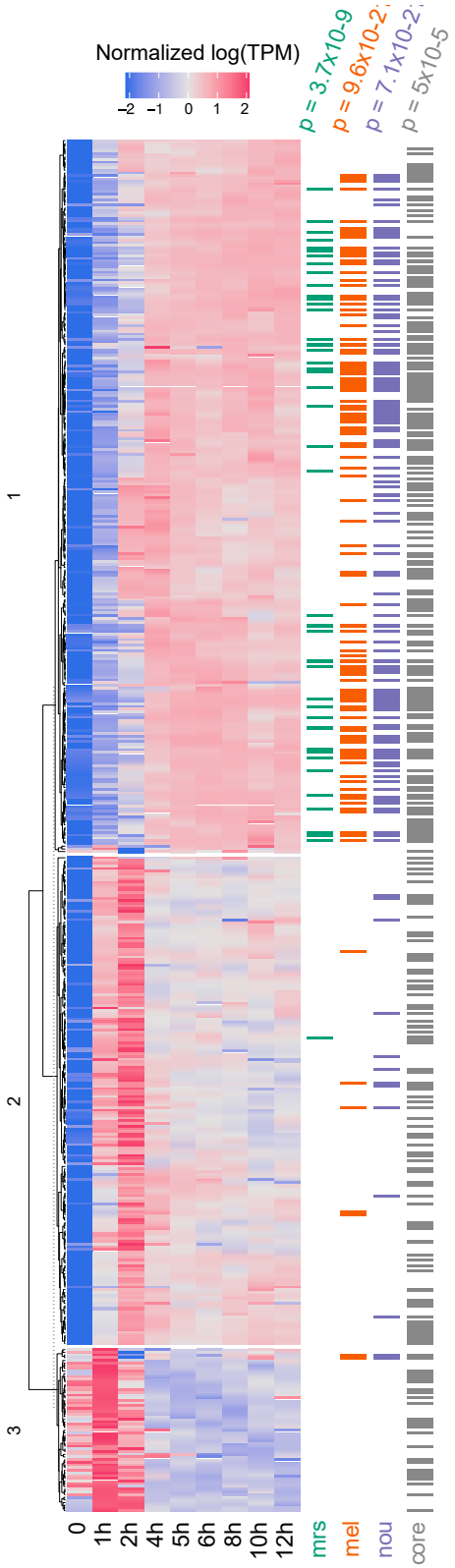

Figure S5. RNA-seq marseillevirus gene expression.
RNA-seq data was generated by [1]. The left part of the figure shows the heatmap of marseillevirus gene expression profiles along a replication cycle. Rows correspond to genes and columns to infection time points in hours (0 h corresponds to 30 min of infection due to virus adsorption period, see [1] for details). Expression data in TPM values were normalized and scaled (see Materials and Methods for details). The clustering was done using a spearman correlation distance and separated in 3 main clusters using kmeans. Genes whose protein products were identified in marseillevirus (mrs in green) [2], melbournevirus (mel in orange) [3] and noumeavirus (nou in blue) [3] are shown at the right of the figure. For the proteomic datasets of noumeavirus and melbournevirus virion particles we displayed the orthologous marseillevirus genes. The gray column represents the core genes. The p-values on the top were calculated using a chi-square test based on the counts among the three clusters.

1. Rodrigues, R.A.L.; Louazani, A.C.; Picorelli, A.; Oliveira, G.P.; Lobo, F.P.; Colson, P.; La Scola, B.; Abrahão, J.S. Analysis of a Marseillevirus Transcriptome Reveals Temporal Gene Expression Profile and Host Transcriptional Shift. *Front Microbiol* **2020**, *11*, 651, doi:10.3389/fmicb.2020.00651.
2. Boyer, M.; Yutin, N.; Pagnier, I.; Barrassi, L.; Fournous, G.; Espinosa, L.; Robert, C.; Azza, S.; Sun, S.; Rossmann, M.G.; et al. Giant Marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 21848–21853, doi:10.1073/pnas.0911354106.
3. Fabre, E.; Jeudy, S.; Santini, S.; Legendre, M.; Trauchessec, M.; Couté, Y.; Claverie, J.-M.; Abergel, C. Noumeavirus replication relies on a transient remote control of the host nucleus. *Nat Commun* **2017**, *8*, 15087, doi:10.1038/ncomms15087.

Computer code

Python code used for cumulative GC-skew and AT-skew calculations

```
#!/bin/python

import sys
import numpy as np
import argparse
import os
parser = argparse.ArgumentParser(formatter_class=argparse.ArgumentDefaultsHelpFormatter)

parser.add_argument('-f', action="store", dest="in_path", default= None, help="Genome file
(.fasta) [REQUIRED]")

args = parser.parse_args()

if args.in_path is None:
    print("Genome file (.fasta) [REQUIRED]")
    exit()

fullinputpath = os.path.abspath(args.in_path)
output_prefix = os.path.split(fullinputpath)[1]
output_prefix = os.path.splitext(output_prefix)[0]

'''Opening Fasta'''
fasta = open(fullinputpath, "r")
fasta.readline()                              #get rid of header
sequence = fasta.read().replace("\n","")    #removes \n for counting

'''init'''
compteur_A = 0
compteur_T = 0
compteur_G = 0
compteur_C = 0
boucle_A = 0
boucle_T = 0
boucle_G = 0
boucle_C = 0
GCskew = []
ATskew = []
AGskew = []
ACskew = []
TGskew = []
TCskew = []
calculGC = 0
calculAT = 0
calculAG = 0
calculAC = 0
calculTG = 0
calculTC = 0
pas = 100                                     #step size
window=1000                                   #windowsize of N nucleotides
taille = int((len(sequence)/pas)+1)
cumul=np.empty((taille, 4))


'''Boucle'''
for start in range(0,len(sequence),pas):    #browse the genome
    end=start+window
    if end > len(sequence):
        end = len(sequence)
    for i in range(start,end):              #count in groups of N nucleotides
        if sequence[i]=="A" :
            boucle_A+=1
        if sequence[i]=="T" :
            boucle_T+=1
        if sequence[i]=="G":
```

```
            boucle_G+=1
        if sequence[i]=="C":
            boucle_C+=1
    if boucle_A==0 :
        boucle_A=0.1
    if boucle_T==0 :
        boucle_T=0.1
    if boucle_C==0 :
        boucle_C=0.1
    if boucle_G==0 :
        boucle_G=0.1
    #cumulative GC and ATskews
    calculGC += ((float(boucle_G)-float(boucle_C))/(float(boucle_G)+float(boucle_C)))
    calculAT += ((float(boucle_A)-float(boucle_T))/(float(boucle_A)+float(boucle_T)))
    GCskew.append(calculGC)
    ATskew.append(calculAT)

    boucle_A = 0                            #set to 0
    boucle_T = 0
    boucle_G = 0
    boucle_C = 0


''' Write & Close - Files'''
fasta.close()
np.savetxt("{}_GCskewscore.csv".format(output_prefix), GCskew, delimiter=",",fmt='%f')
np.savetxt("{}_ATskewscore.csv".format(output_prefix), ATskew, delimiter=",",fmt='%f')
print("Done!\n2 files have been generated : XXskewscore.csv\nThanks for using this script")

'''Check the generated files on R'''
```

Dataset S1

Reassembled nucleotide genome sequence of tokyovirus (fasta file)

```
>tky
ATGACTTCTGTTATCTGTGGCACCCGCGTAACTGCTGCTTCCGGTTTTGTGGATTTGGCGACTTTCTCCGATTTGGAGGC
CTACCTCTATGGTGGTTGTTCTGCCGTCACTTATTTTGTGCGTGCCATCAAAAAGGCTAATTGGTTCTCTTTCCTCCCCG
TCGTTCTCCGCAACATCTCGGGTCTTCCCGGTTTCGGCTCAGAGTTTTCCGCCTCTGTGAATCGTTCTGGCGATTACGTC
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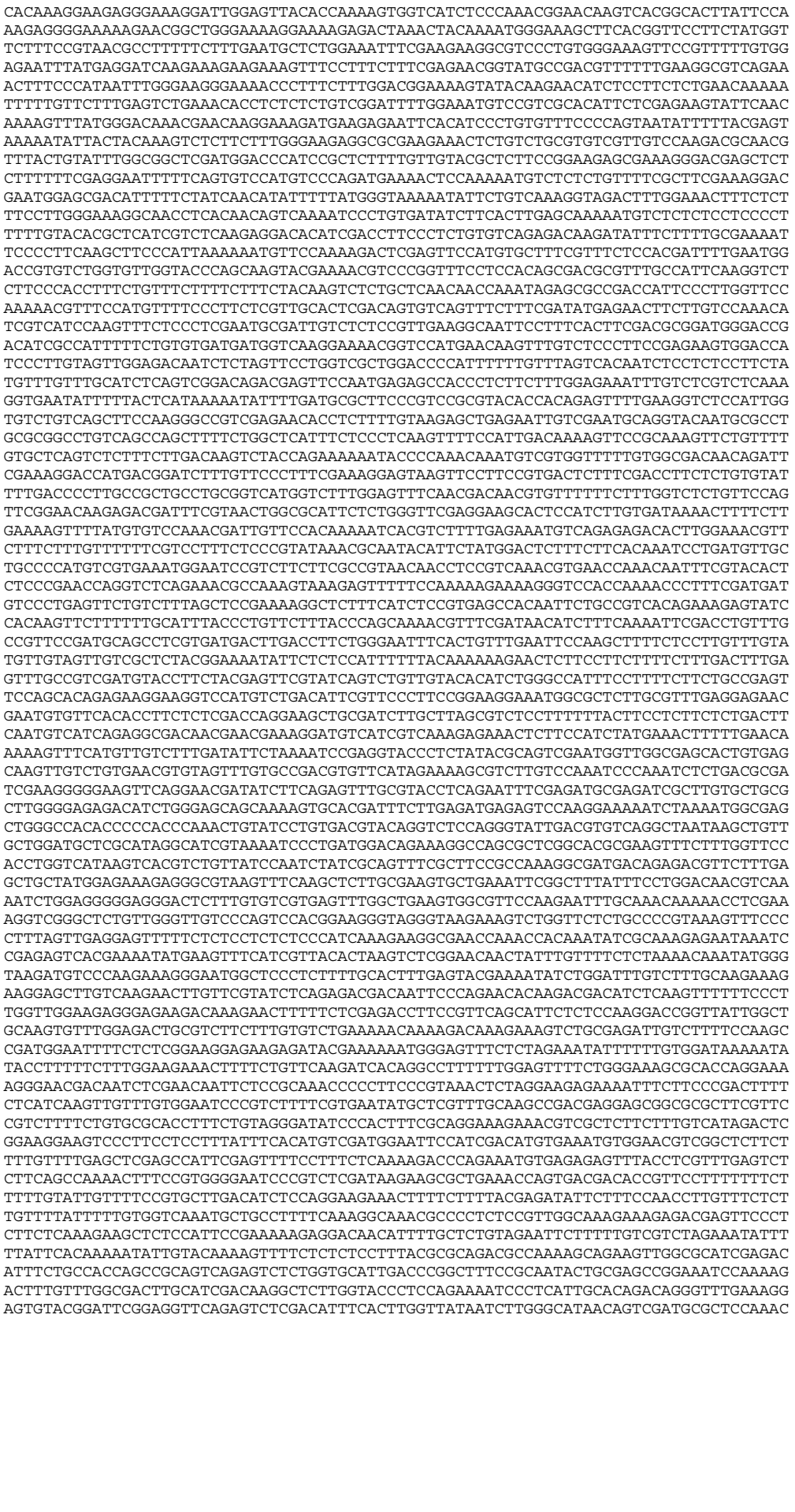

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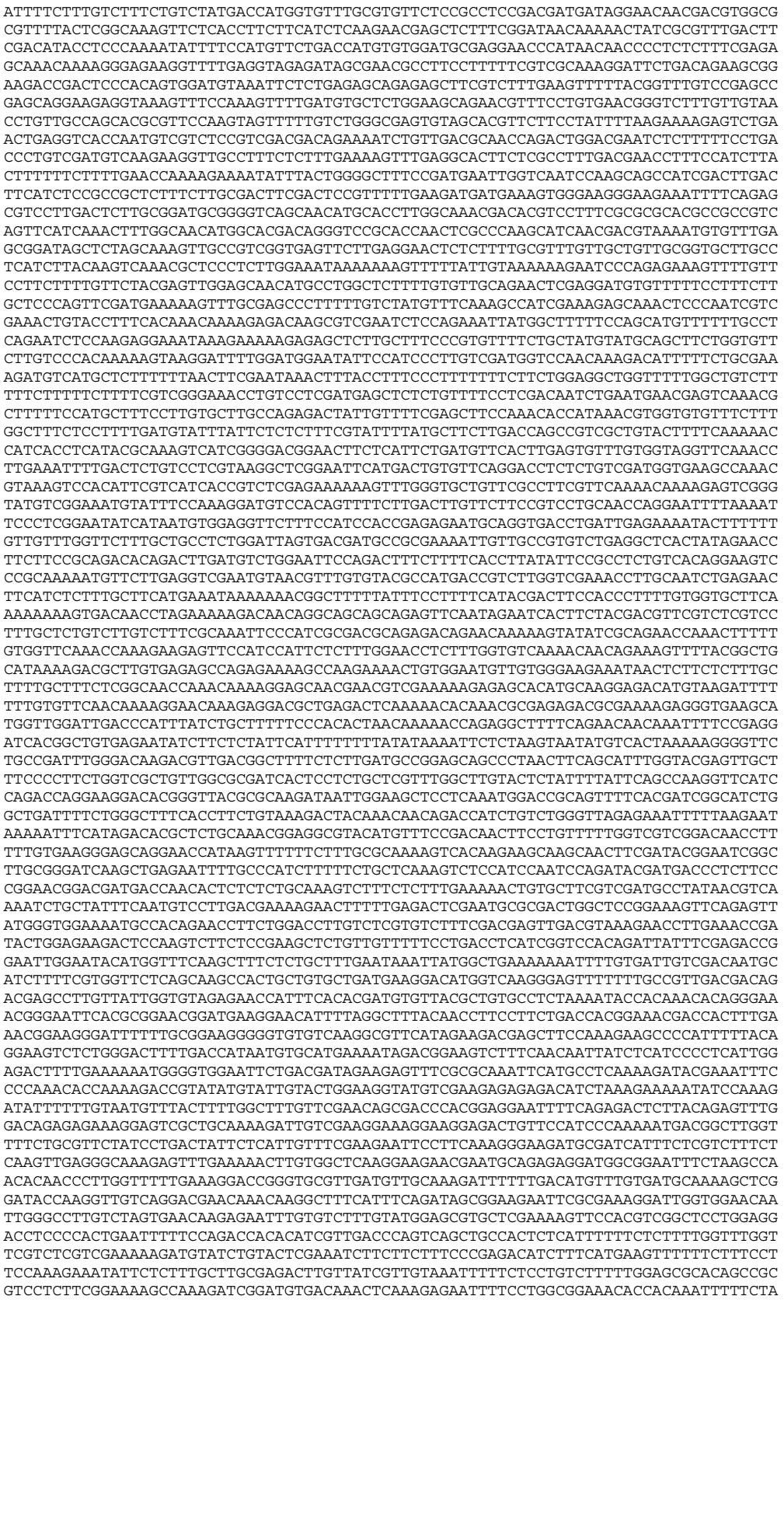

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