Supporting Information

Redrejo-Rodríguez et al. 10.1073/pnas.1216635109

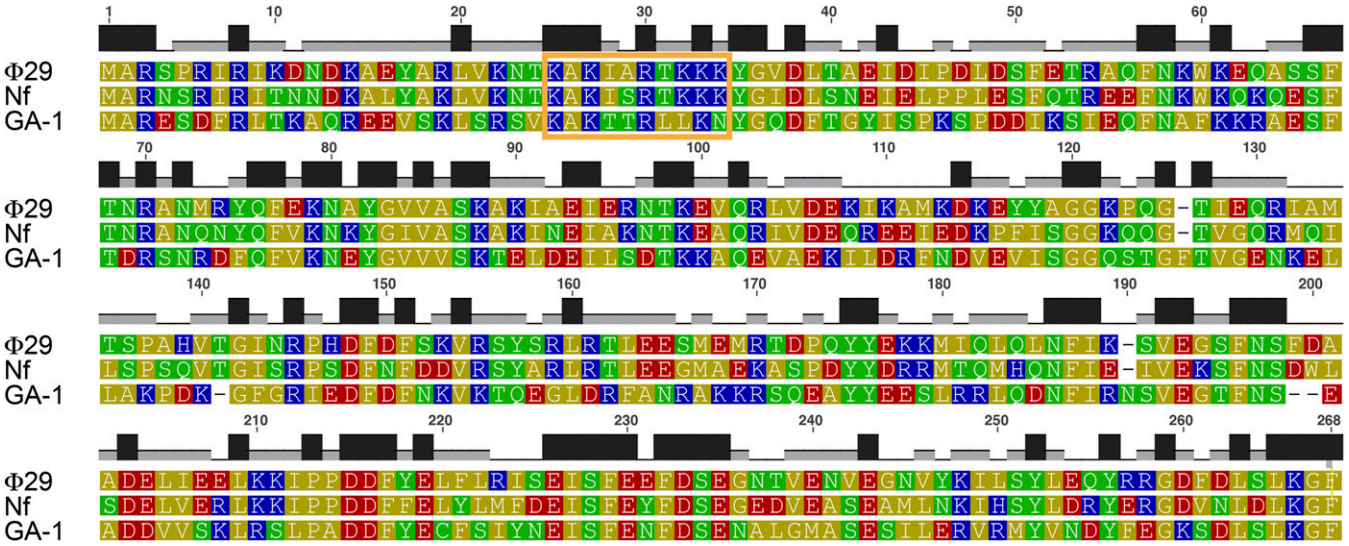

Fig. S1. Alignment of Φ29, Nf, and GA-1 TP sequences. A multiple sequence alignment was carried out with ClustalW. Residues are colored according to polarity, with positive, negative, hydrophobic, and neutral residues in blue, red, olive green, and light green, respectively. Above the sequences, the relative similarity of residues at each position is represented with gray and black bars. The orange box marks the amino acids portion that was predicted as NLS in Φ29 and Nf sequences but not in GA-1.

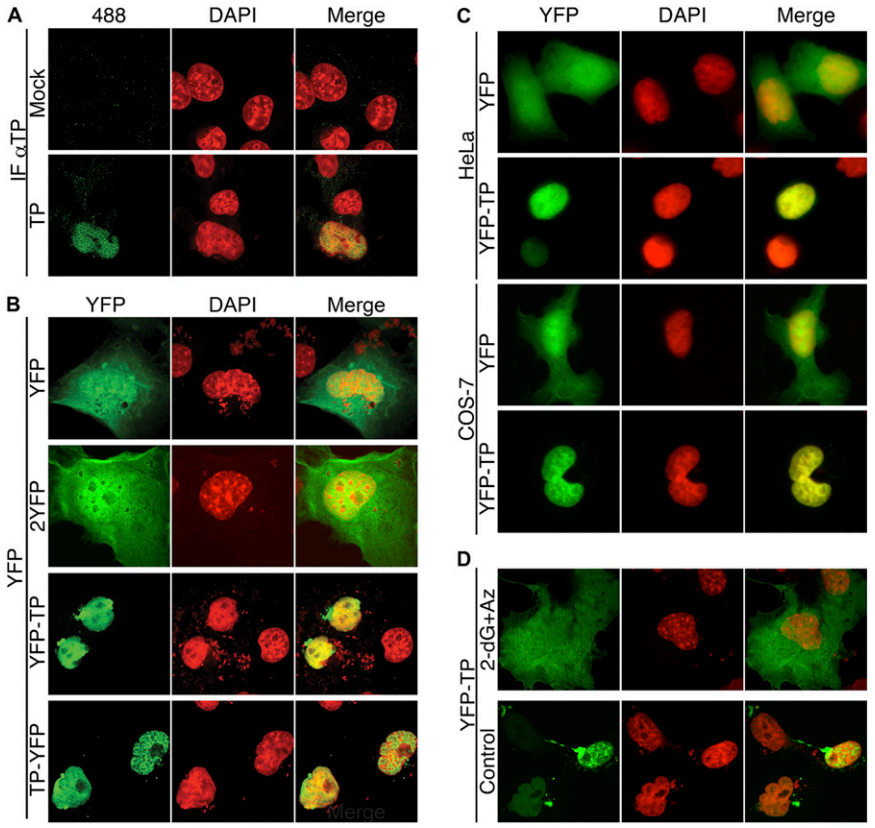

Fig. S2. Different Φ29 TP constructions localize in the eukaryotic cell nucleus. (*A*) Confocal images of immunofluorescence of COS-7 cells mock-transfected and transfected with a plasmid that expresses the Φ29 TP (indicated as TP). Immunofluorescence was carried out as in (1), with specific antibodies raised against Φ29 TP and visualized with secondary alexa 488 (green channel). (*B*) Confocal images of COS-7 cells expressing the indicated YFP, 2YFP, YFP-TP, and TP-YFP fusions. (*C*) Fluorescent images of HeLa and COS-7 cells expressing the indicated YFP and YFP-TP proteins. Fluorescence was detected with a dual CFP/YFP-ET filter (89002; Chroma) and imaging acquisition was performed as described using a Sony CoolSnap HQ cooled CCD camera (Roper Scientific) attached to a Zeiss Axiovert 200M microscope. (*D*) Confocal images of COS-7 cells that expressed YFP-Φ29TP fusion after energy depletion treatment with 2-deoxyglucose and azide (2-dG+Az) to block active nuclear internalization mechanisms. For clarity, YFP and DAPI fluorescent signals are false-colored green and red, respectively.

1. Redrejo-Rodríguez M, García-Escudero R, Yáñez-Muñoz RJ, Salas ML, Salas J (2006) African swine fever virus protein pE296R is a DNA repair apurinic/apyrimidinic endonuclease required for virus growth in swine macrophages. *J Virol* 80(10):4847–4857.

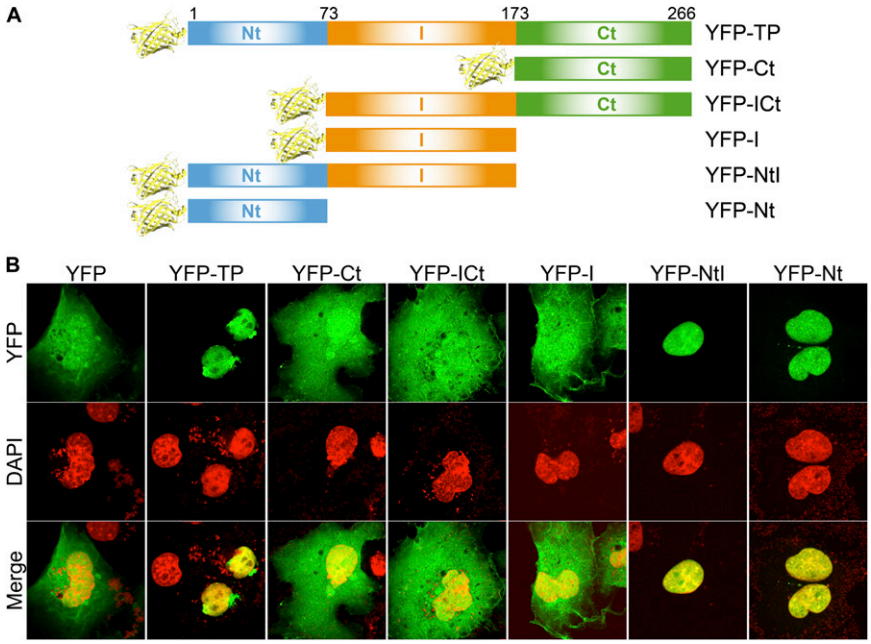

Fig. S3. Nuclear targeting of Φ29 TP domains. (*A*) Schematic representation of the YFP fusions to Φ29 TP and the three functional and structural domains: (*i*) the C-terminal domain that contains the serine-232 priming residue, (*ii*) the intermediate domain that contributes to the interaction with the DNA polymerase, and (*iii*) the Nt that is required for DNA binding and nucleoid association (1). (*B*) YFP, DAPI staining, and merge confocal images of COS-7 cells expressing YFP and YFP-fusions to wild-type Φ29 TP and TP domains. For clarity, YFP and DAPI fluorescent signals are false-colored green and red, respectively.

1. Muñoz-Espín D, Holguera I, Ballesteros-Plaza D, Carballido-López R, Salas M (2010) Viral terminal protein directs early organization of phage DNA replication at the bacterial nucleoid. *Proc Natl Acad Sci USA* 107(38):16548–16553.

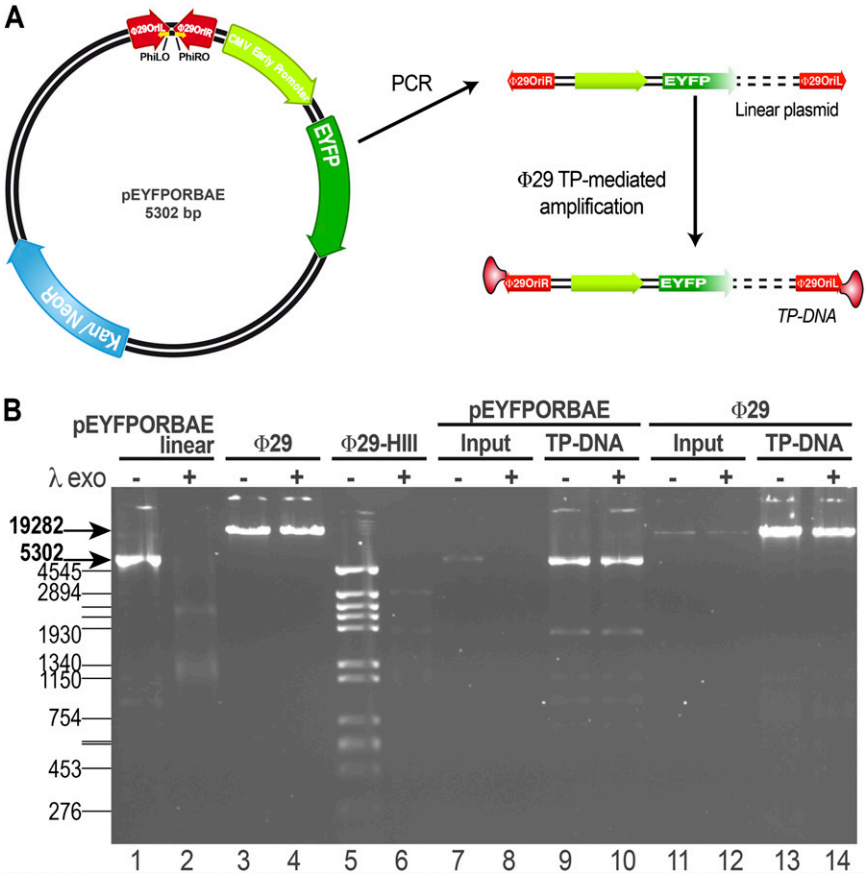

Fig. S4. In vitro generation of pEYFPORBAE plasmid with covalently linked Φ29 TP. (*A*) The pEYFPORBAE plasmid contains fused fragments from the left and right ends of the Φ29 genome. Thus, linear DNA molecules with the Φ29 genome ends can be generated by PCR amplification of the plasmid using the PhiRO and PhiLO primers (Table S2), whereas TP-mediated amplification of this linear DNA with the Φ29 minimal replication system gives rise to DNA molecules that contain a TP linked at each 5′ end (1). (*B*) Agarose gel that shows differences between PCR and TP-mediated amplification. Samples were treated with proteinase K, phenol-extracted, and when indicated, subsequently digested with λ exonuclease (New England Biolabs). The pEYFPORBAE plasmid amplified by PCR (lanes 1 and 2) and the Φ29 DNA HindIII restriction fragments (lanes 5 and 6) are fully degraded by the exonuclease treatment. On the other hand, proteinase K digestion of Φ29 TP-DNA degrades the TP protein, leaving a small peptide (2). Accordingly, Φ29 TP-mediated amplification (lanes 9 and 10) gave rise to exonuclease-resistant DNA molecules. Φ29 genome purified from infected bacteria (3) and amplified in vitro (lanes 11–14) are also resistant to exonuclease digestion. The electrophoretic migration of pEYFPORBAE, Φ29 DNA, and Φ29 DNA HindIII restriction fragments are indicated on the left (in bp).

1. Mencía M, Gella P, Camacho A, de Vega M, Salas M (2011) Terminal protein-primed amplification of heterologous DNA with a minimal replication system based on phage Φ29. *Proc Natl Acad Sci USA* 108(46):18655–18660.
2. Hermoso JM, Méndez E, Soriano F, Salas M (1985) Location of the serine residue involved in the linkage between the terminal protein and the DNA of phage Φ29. *Nucleic Acids Res* 13 (21):7715–7728.
3. Peñalva MA, Salas M (1982) Initiation of phage Φ29 DNA replication in vitro: Formation of a covalent complex between the terminal protein, p3, and 5′-dAMP. *Proc Natl Acad Sci USA* 79(18):5522–5526.

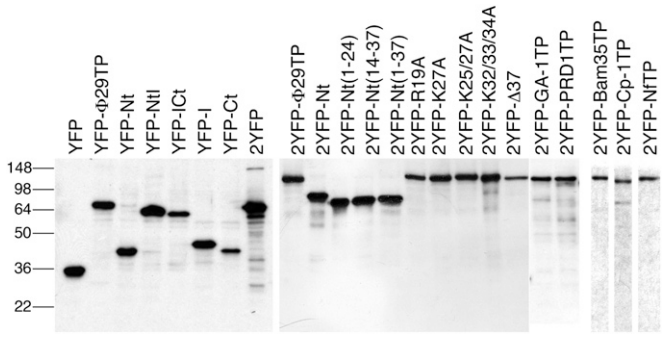

Fig. S5. Expression and stability of YFP fusion proteins in COS-7 cells. Western blot analysis of YFP and all YFP or 2YFP-TP fusions expressed in cell extracts from transfected COS-7 cells. Electrophoretic migration of molecular size markers in kDa is indicated on the left.

Table S1. Summary of predictions and determinations of NLSs in TPs from diverse origins

Phage	Virus family	Host	TP accession no.	NLStradamus	PSORT	Nuclear
Φ29	*Podoviridae*	*Bacillus subtilis*	P03681.1	25–34*	—	+
GA-1	*Podoviridae*	*Bacillus subtilis*	NP_073686.1	—	—	–
Nf	*Podoviridae*	*Bacillus subtilis*	ACH57070.1	25–34*	—	+
Cp-1	*Podoviridae*	*Streptococcus pneumoniae*	NP_044816.1	222–230	5–11[†] 17–20[†] 223–227[†]	+
Av-1	*Podoviridae*	*Actinomyces* sp.	YP_001333658	351–360	195–199[†]	n.d.
ΦCP24R	*Podoviridae*	*Clostridium perfringens*	AEW47836.1	4–18 69–98	77–100[‡]	n.d.
asccΦ28	*Podoviridae*	*Lactococcus lactis*	ACA21480.1	12–16*	9–15[‡]	n.d.
Bam35	*Tectiviridae*	*Bacillus thuringiensis*	NP_943750.1	4–55	14–31[‡] 214–220[†]	+
PRD1	*Tectiviridae*	*Escherichia coli*	P09009.1	243–259	3–6[†] 25–41[‡]	+
ΦYS40	*Myoviridae*	*Thermus termophilus*	YP_874078.1	—	—	n.d.
ABV	*Ampullaviridae*	*Acidianus* sp.	A4ZU93.1	—	—	–

Predictions were carried out with NLStradamus (www.moseslab.csb.utoronto.ca/NLStradamus) and PSORT (http://psort.hgc.jp/form2.html) Internet servers, using the amino acid sequence of TPs from representative phages from diverse families and hosts. For reference, the GeneBank accession number of each TP is presented. PNLSs and positions from each server output are indicated. A NLStradamus pNLS search was carried out with a cutoff value of 0.75 (or 0.45 where indicated with *). PSORT server predicted monopartite ([†]) or bipartite ([‡]) NLSs. As detailed in the text, nuclear localization for selected TPs was also experimentally verified for a selected group of representative TPs and positive (+) or negative (–) results are shown on the right. n.d., not determined.

Table S2. List of oligonucleotides used for PCR amplification

Name	Amplified insert(s)	Sequence (5′–3′)
5	TP, Nt, NtI, Nt(1–24) & Nt(1-37)	AGATAGAATTCTATGGCGAGAAGTCCACG
6	TP, ICt, and Ct	TCCTTGGATCCTTATTAGAACCCCTTTAAGCTTAGATC
1	YFP	CCGGTGAATTCTATGGTGAGCAAGGGCGAGG
2	YFP	CGAGAGGATCCTTATTACTTGTRACAGCTCGTCCATGCCG
11	Nt(14–37) and Nt(1–37)	ATTTCGGATCCTTATTATACACCATACTTTTTCTTCGTTC
12	I and ICt	AACCGGAATTCTATGCGTTATCAGTTCG
10	Nt	TCGAAGGATCCTTATTAATTAGCACGGTTAGTGAAAG
13	Ct and Ict	AGAACGAATTCTCAGTATTATGAAAAGAAAATG
15	I and NtI	TTCTTGGATCCTTATTAAGGGTCTGTTCTCATCTCC
7	TP	GGAGATGAATTCTGATGGCGAGAAGTCCACG
8	TP	TTAACGGATCCCGGAACCCCTTTAAGCTTAGATC
9	TP	CATCCGGATCCCGTTATTAGAACCCCTTTAAGCTTAGATAAAGTC
16	Nt(1–24)	TCTCGCGGATCCTTATTATGTATTCTTGACCAATCGAGCG
17	Nt(14–37)	GGTATATCAATTTCAGCGAATTCTTATACACCATACTTTTTCTTCG
18	K25/K27	CGATTGGTCAAGAATACAGCAGCCGCGATTGCGAGAACGAAG
19	K25/K27	CTTCGTTCTCGCAATCGCGGCTGCTGTATTCTTGACCAATCG
20	K27A	AAGAATACAAAAGCCGCGATTGCGAGAACGAA
21	K27A	TTCGTTCTCGCAATCGCGGCTTTTGTATTCTT
22	R19A	AAAGCCGAATACGCTGCATTGGTCAAGAATAC
23	R19A	GTATTCTTGACCAATGCAGCGTATTCGGCTTT
26	TP PRD1	CCGGCCGAATTCTATGGCGAAGAAAAAACCAGTAGAA
28	TP GA-1	CCGGCCGAATTCTATGGCAAGAGAGTCAGACTTTAGGCTTACAAAG
27	TP PRD1	TTCTTGGATCCTTATTAAACCCCCTTGCTGCCATAGCCGCGTTTTTG
29	TP GA1	TTCTTGGATCCTTATCAGAAACCCTTTAAACTTAAATCACTCTTTCCCTC
30	ΔN37	CCGGCCGAATTCTATGGACCTTACCGCTGAAATTGATATACCTGACCTTGATTCATTT
3	YFP	CGCGGAATTCTTGTACAGCTCGTCCATGCCGAGAGT
4	YFP	CGCGCTCGAGCTATGGTGAGCAAGGGCGAGGAG
31	TP	GAAGAGCATATGGGCTGGAGCCATCCCCAGTTCGAAAAAGGTTCGCCGGAATTCATGGCAGAAGT
32	TP	GATTAAGCATTGGTAGACGTCAGACCAAGTTT
33	TP Nf	CCGGCCGAATTCTATGGCAAGAAATTCACGTATACGCATTACGA
34	TP CP-1	CCGGCCGAATTCTATGGCTTTAACACCAAAACAAAGGAAG
36	TP Nf	GGCCCGGATCCTTATTAAAACCCCTTTAAGTCAAGATTCACGTC
37	TP CP-1	GTCCCGGATCCTTATCACTTCTTCCCTCGCTTCTTTCGTCTTCTCAT
35	TP Bam35	CCGGCCGAATTCTATGGCAAATAAACGGTTAAAGAAGAAAC

Table S2. Cont.

Name	Amplified insert(s)	Sequence (5′–3′)
38	TP Bam35	GTCCCGGATCCTTATTAGTAGTAGTCATCATTATCCCAACTTTC
PhiLO	pEYFPORBAE	^{P}AAAGTAAGCCCCCACCCTCACATGATACCA
PhiRO	pEYFPORBAE	^{P}AAAGTAGGGTACAGCGACAACATACACCAT

The target insert amplified with each oligonucleotide is indicated. For simplification, Φ29 TP is indicated as TP and Φ29 TP domains are also specified in abbreviated names (ct, C-terminal domain; I, intermediate domain; Nt, N-terminal domain); ^{P}, 5′ phosphate group.