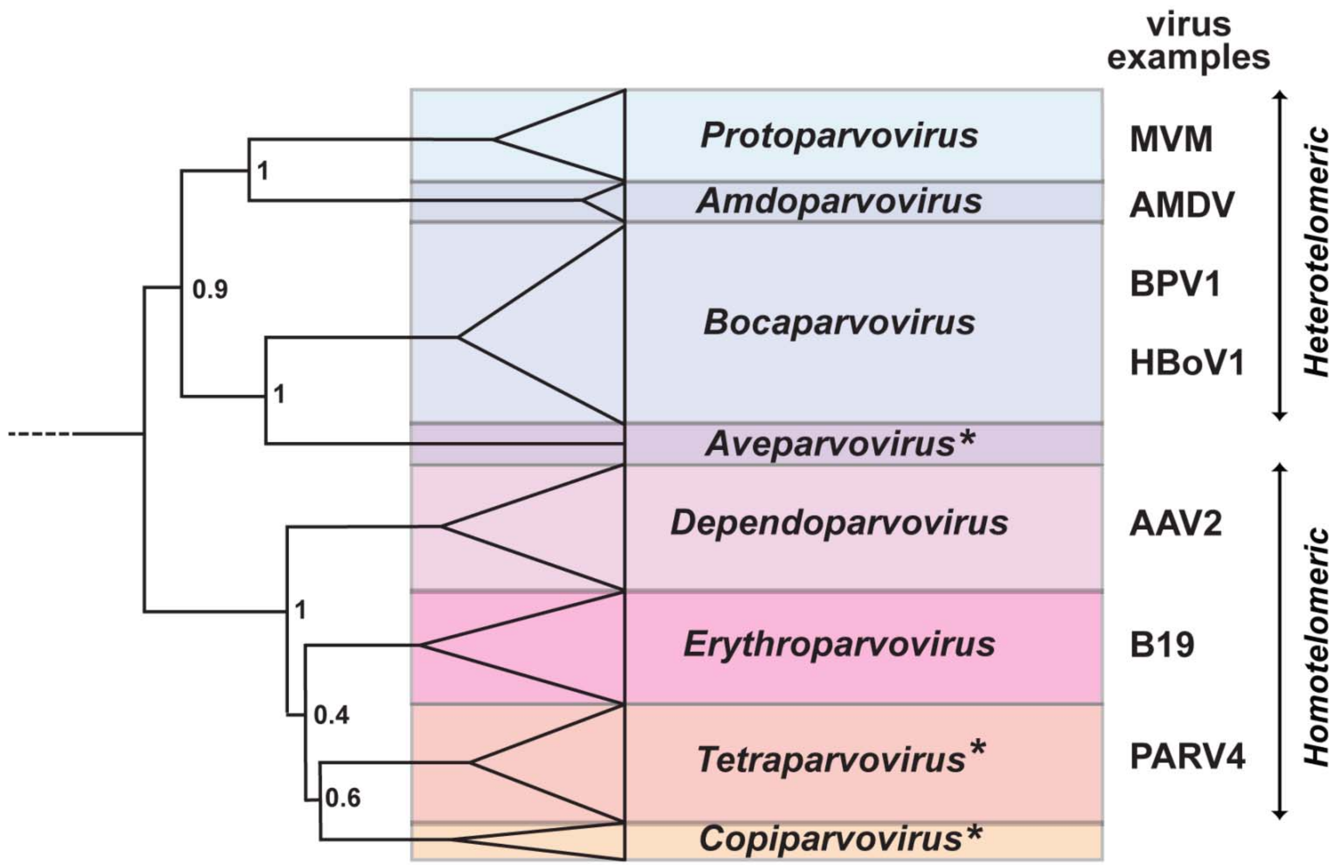
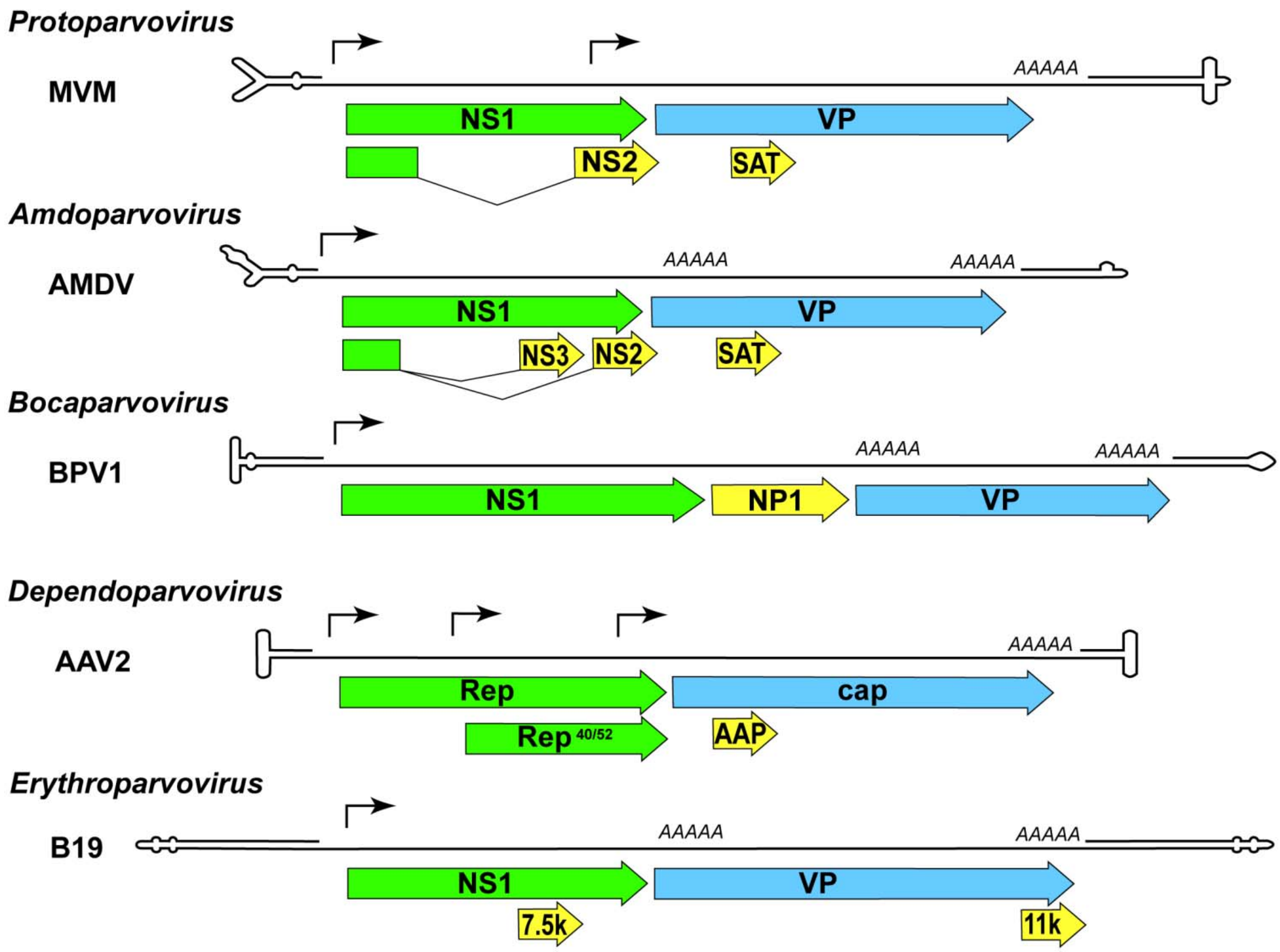
Supplemental Figure 1.



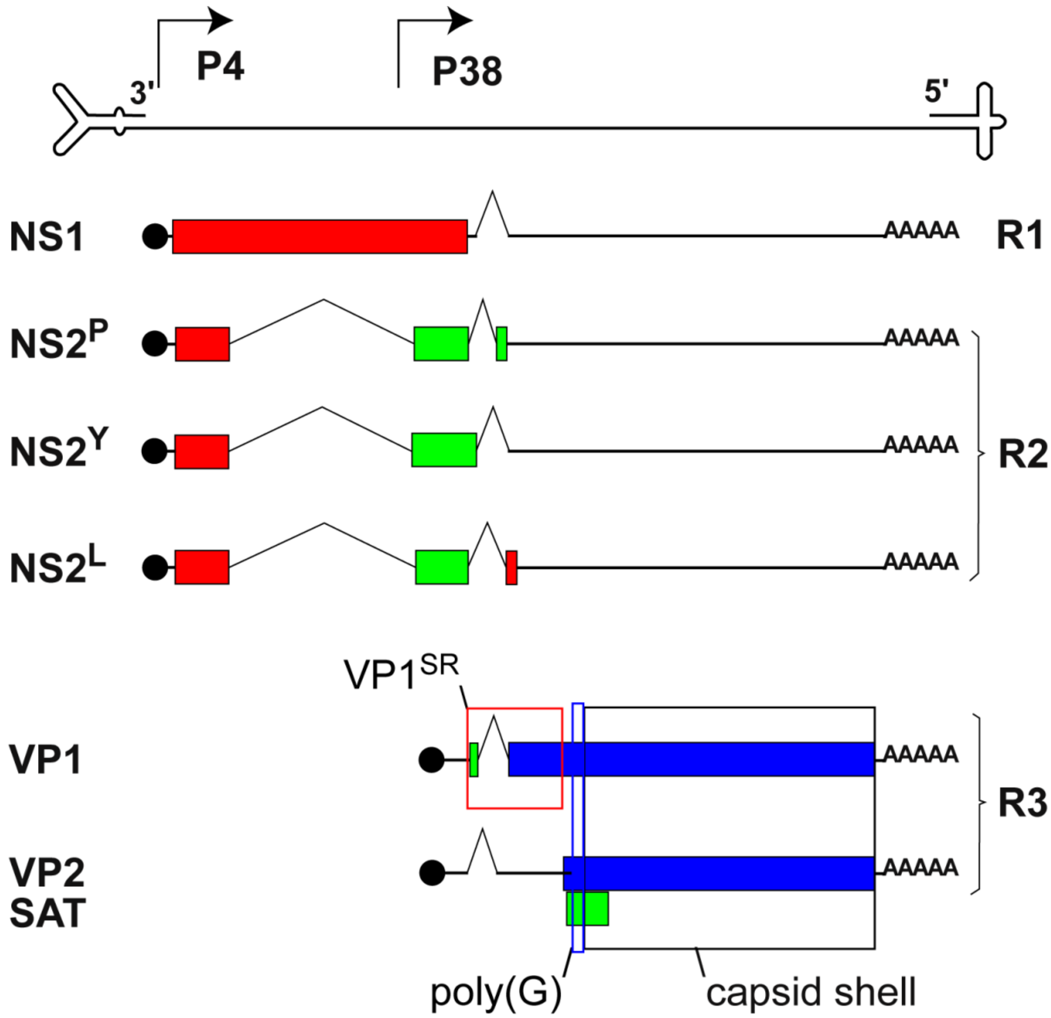
Phylogenetic tree showing genera in the Parvovirinae, a subfamily of the family Parvoviridae that includes all viruses infecting vertebrate hosts. Phylogenetic analysis is based on the amino acid sequence of the viral replication initiator protein, NS1. The size of the color block for each genus indicates the relative number of species currently recognized, as an indicator of its diversity. Along with other shared characteristics, all viruses in a genus are monophyletic and encode NS1 proteins that are generally >30% identical to each other at the amino acid sequence level but <30% identical to those of other genera, whereas within a species these proteins show >85% amino acid sequence identity and diverge by >15% from viruses in other species. Bayesian trees were calculated using BEAST, with a Yule model of speciation and an exponential relaxed molecular clock (14). Trees were viewed in FigTree in ultrametric format on an arbitrary scale, midpoint rooted, and with posterior probability scores indicated at significant nodes. Asterisks denote the names of new genera. Virus examples: MVM, minute virus of mice (J02275); AMDV, Aleutian mink disease virus (JN040434); BPV1, bovine parvovirus 1 (DQ335247); HBoV1, human bocavirus 1 (JQ923422); AAV2, adeno-associated virus 2 (AF043303); B19, parvovirus B19 (M13178); PARV4, human parvovirus 4 (AY622943).

Supplemental Figure 2.



Genetic strategies of representative viruses from five genera in the subfamily Parvovirinae. Genomes from viruses within the type species of each genus are shown as a single line terminating in hairpin structures. These are minute virus of mice (MVM), Aleutian mink disease virus (AMDV), bovine parvovirus 1 (BPV1), adeno-associated virus 2 (AAV2), and parvovirus B19 (B19). The hairpins, drawn to represent their predicted structures, are scaled approximately 20× relative to the rest of the genome. Major open reading frames encoding proteins are represented by arrowed boxes, some of which are linked by splicing to encode specific ancillary proteins. Proteins are shaded green for the major SF3 domain–containing replication initiator protein (called NS1 in most genera, but Rep in *Dependoparvovirus*), blue for the structural (VP) proteins of the capsid, and yellow for sequences unique to the ancillary nonstructural proteins (NS2 and NS3 also share a common upstream intron with NS1). Transcriptional promoters are indicated by solid arrows and polyadenylation sites by the AAAAA sequence block. Abbreviations: SAT, small alternatively translated protein; NP1, nuclear protein 1; AAP, assembly-activating protein.

Supplemental Figure 3.



Transcription and coding profile of minute virus of mice (MVM). The viral genome is indicated by a single line terminating in disparate hairpin telomeres (scaled at ~20× relative to the rest of the genome). Below this, line diagrams represent individual transcripts. Coding sequences are indicated in these mRNAs by boxes, which are color coded to indicate which of the three reading frames they employ. Transcriptional promoters are positioned at map units 4 and 38 (P4, P38). P4 is the first promoter to fire during infection and drives the synthesis of transcripts R1 and R2, which are polyadenylated (AAAAA) near the right end of the genome. These encode NS1 and three C-terminally distinct forms of NS2 ($NS2^{P}$, $NS2^{Y}$, and $NS2^{L}$). NS1 then binds upstream of the P38 promoter and drives synthesis of R3 transcripts that are alternatively spliced to encode either VP1 or VP2 and SAT. N-terminal protein sequences boxed in red, denoted $VP1^{SR}$ (VP1-specific region), are unique to VP1. Sequences boxed in black, comprising the C-terminal region common to all VP polypeptides, assemble to form the capsid shell; poly(G), boxed in yellow, identifies a short glycine-rich peptide sequence present in all VPs that can be modeled into X-ray density occupying capsid fivefold pores in DNA-containing virions.

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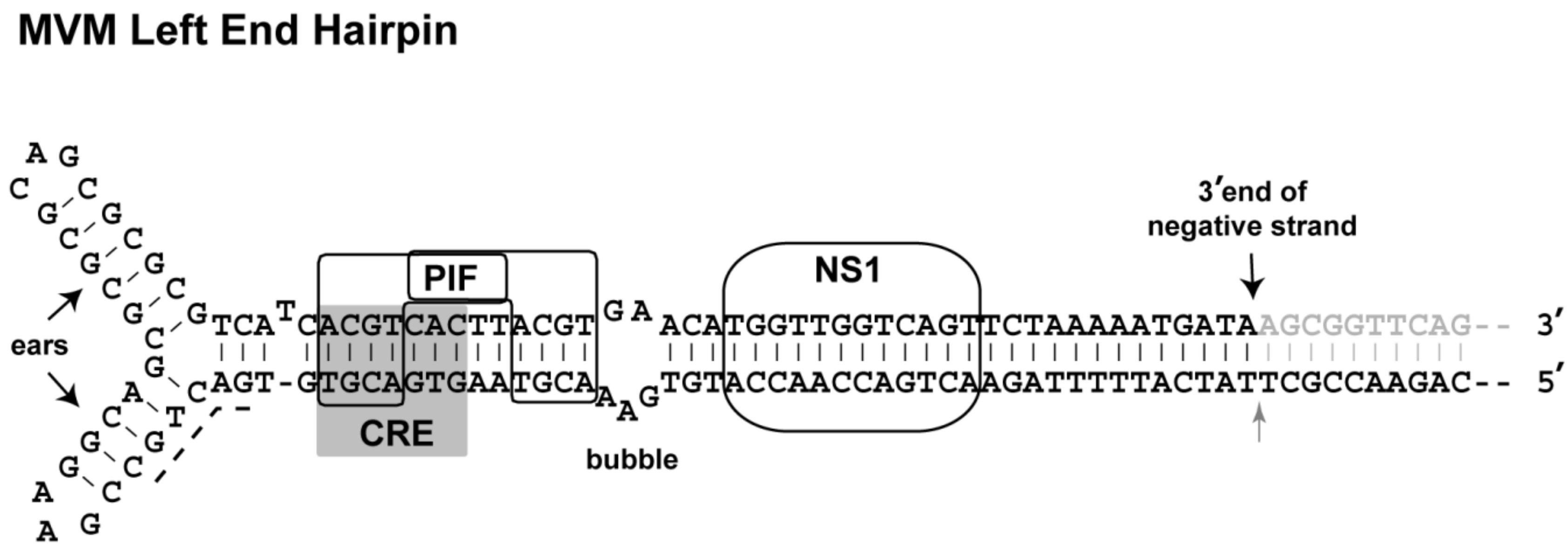
Supplemental Figure 4.



Sequence and predicted structure of the minute virus of mice (MVM) left-end hairpin. The 121-nt left-end telomere of the negative-sense MVM genome (*black letters*) is predicted to fold into a Y-shaped hairpin containing small axial palindromes that form the ears and a 43-bp duplex stem region that is interrupted by a mismatched bubble sequence, where the triplet 5′-GAA-3′ on the inboard arm of the hairpin in virion DNA is opposed by the doublet 5′-GA-3′ on the outboard arm. Viral DNA synthesis is initially primed from the 3′ nucleotide of this hairpin (*vertical black arrow*), to generate a duplex molecule (*gray letters*) in which the two strands are linked to each other through the hairpin. The binding site that positions NS1 on this structure is boxed, separated by the bubble from the binding site for its essential cellular cofactor PIF (parvovirus initiation factor), which consists of two half sites (5′-ACGT-3′) where the distal site overlaps with a CRE (cAMP response element) involved in P4 transcription. The origin sequence cannot be nicked in this hairpin orientation, but its potential nick site (*vertical gray arrow*) is active in multimeric duplex forms of the viral genome.

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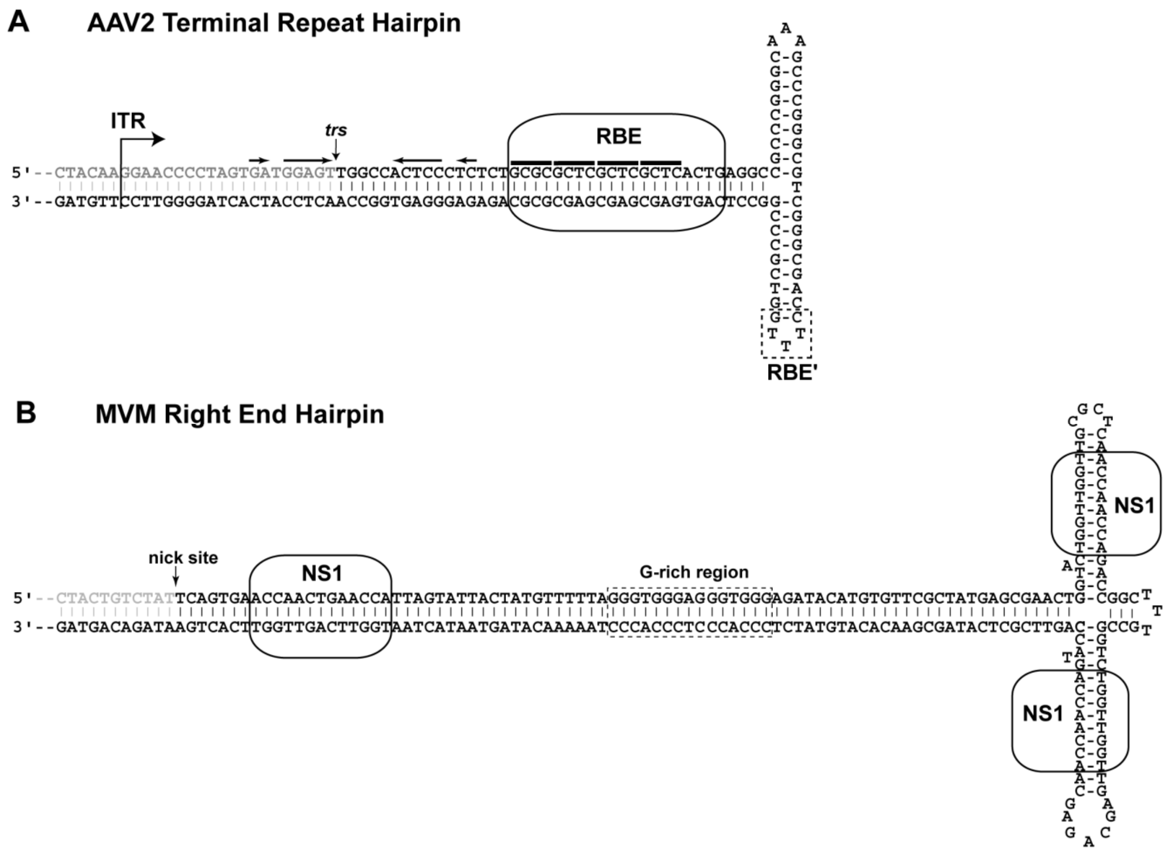
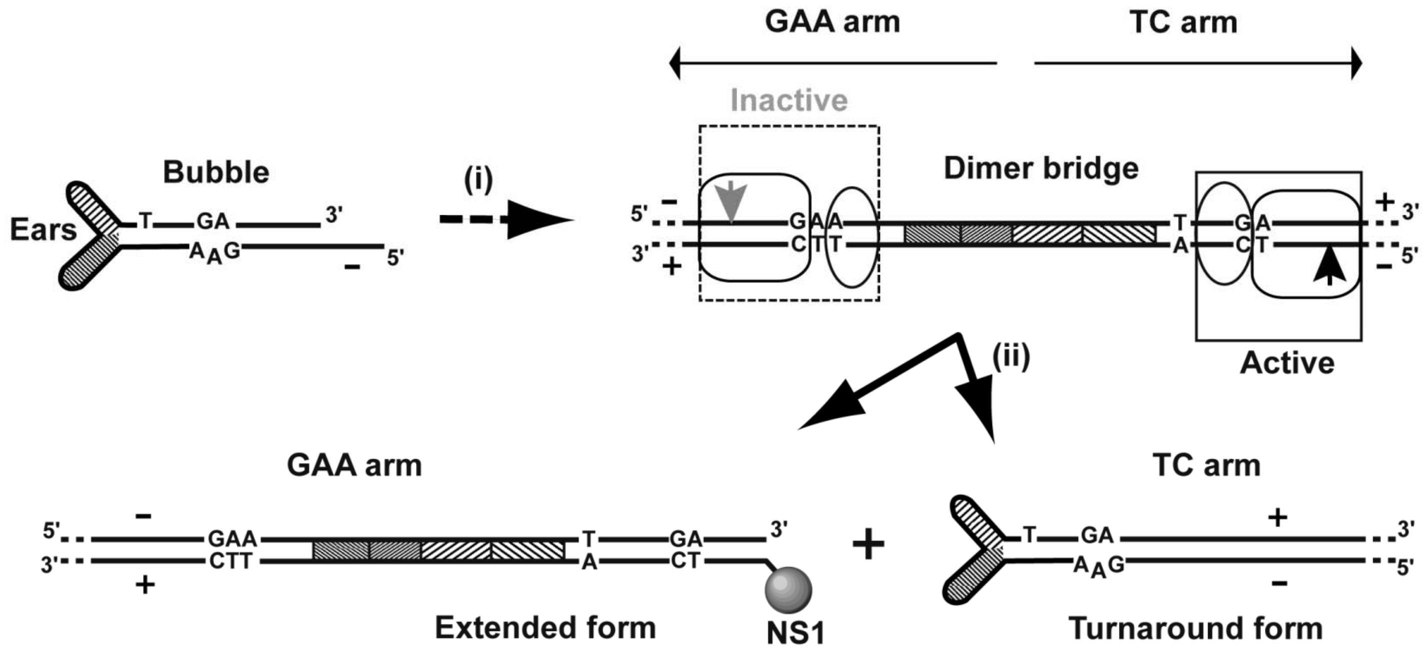
Supplemental Figure 5.



(*a*) Sequence and predicted structure of adeno-associated virus 2 (AAV2) hairpin termini. The 125-nt hairpins of AAV (*black letters*) have internal axial palindromes that create a T-shaped structure. In AAV, and other homotelomeric viruses, these hairpins form part of an inverted terminal repeat. The AAV2 replication initiator protein, Rep$^{68/78}$, binds to five partially degenerate tandem copies of the motif 5′-GAGC-3′, labeled as the RBE (Rep-binding element) (*box*), which positions it to introduce a single-strand nick (*vertical arrow*) at a site labeled trs (terminal resolution site). This site is flanked by short palindromic repeats that can rearrange into a stem-loop structure that promotes nicking by presenting the trs as a single strand. An additional recognition sequence, RBE′ (5′-CTTTG-3′) (*dashed box*), positioned at the tip of the hairpin arm opposite the nick site also promotes, but is not absolutely essential for, nicking.

(*b*) Sequence and predicted structure of the right-end hairpin of MVM. The 248-nt right-end telomere of minute virus of mice (MVM) (*black letters*) can switch between the cruciform structure illustrated here, in which a 36-bp palindrome containing reiterations of the NS1-binding motif, 5′-TGGT-3′ (*box*), folds into two axial arms, or a fully extended duplex form, in which only a few nucleotides are unpaired. NS1 also binds the boxed 5′-TGGT-3′ motifs in the hairpin stem that positions it over the nick site (*vertical black arrow*). For nicking to occur, DNA-bending proteins from the HMGB family must coordinate interactions between the NS1 complexes bound at each end of the hairpin stem, creating an ~30-bp double-helical loop centered on the intervening G-rich DNA (*dashed box*).

Supplemental Figure 6



Junction resolution reaction for the left-end of minute virus of mice (MVM). (*i*) The single, flip, orientation of the left-end hairpin in virion DNA is organized after being unfolded and copied during replication to create the dimer junction sequence that bridges adjacent genomes in replicative form DNA. Cross-hatched boxes represent the palindromic sequences that form the ears of the hairpin form. The sequence derived from the outboard arm of the original hairpin now has a duplex copy of the 5′-GA-3′ bubble dinucleotide, paired with its complement 5′-TC-3′, and serves as an active origin. However, the equivalent region from the inboard arm, which contains the bubble triplet 5′-GAA-3′ paired with its complement 5′-TTC-3′, is inactive, because the extra nucleotide prevents essential interactions between NS1 (bound at the square boxes) and PIF (parvovirus initiation factor) (bound at the ovals) that are required for activation of the nicking complex. As a result, the junction is resolved asymmetrically by a protracted resolution reaction that conserves the single, flip, sequence organization in progeny strands. This involves nicking the TC arm origin (*vertical black arrow*), followed by unidirectional synthesis, reconfiguration through a heterocruciform intermediate, and the melting and reorientation of both arms. (*ii*) The telomeric products of this reaction observed in vivo; products of the GAA arm emerge as extended-form duplexes, whereas those of the TC arm contain a single copy of the hairpin in the original turnaround configuration.

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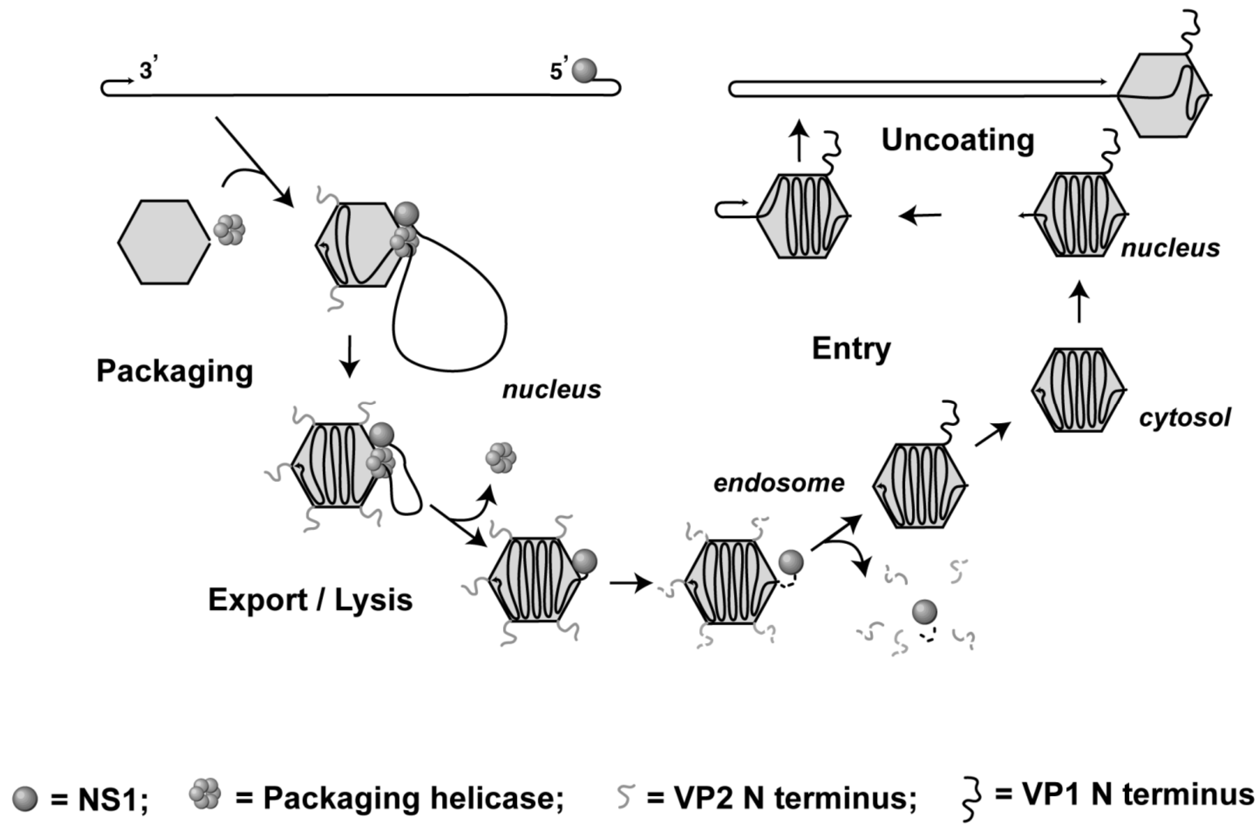
Supplemental Figure 7.



A two-portal model for genome packaging and uncoating (85). A newly displaced progeny single-stranded genome (*top left*) is represented by a single line, drawn 3′ to 5′, left to right, with a shaded circle representing a covalently bound NS1 molecule. The DNA is packaged into a preassembled capsid in a 3′-to-5′ direction, via a capsid portal at one of the icosahedral fivefold vertices. DNA packaging is driven by the helicase activity of a portal-associated NS1 peptide oligomer (depicted as a hexameric structure, by analogy with other SF3 helicases), with the 3′ end of the entering strand lodging near the capsid shell, proximal to its destined exit pore. The rest of the strand is then pumped into the particle under increasing pressure, adopting a topology that will allow it to be subsequently unraveled and ejected in a 3′-to-5′ direction. NS1 is removed from the 5′ end of the DNA during cell entry. Ca^{2+}/Mg^{2+} ions in the virion stabilize the pressurized structure, but when these are depleted in vitro, perhaps mimicking a specific in vivo host factor trigger, the 3′ end of the DNA, followed by the coding sequences, is ejected through a second fivefold pore. This uncoated DNA can support complementary strand synthesis, while its 5′ end remains associated with the capsid.