Expanded View Figures

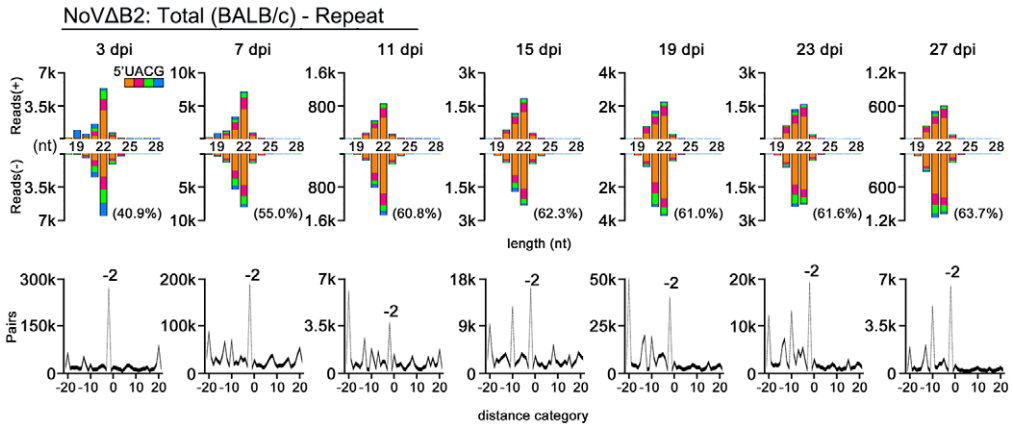

Figure EV1. Long maintenance of vsiRNAs in neonatal mice.

Properties of viral siRNAs (per million mature miRNAs) cloned and sequenced from the time course series of NoVΔB2-infected BALB/c suckling mice (a repeat of Fig 1F). Size distribution, 5' terminal nucleotide, and duplexes by 22-nt vsiRNAs are indicated. The 1 U% of 21- to 23-nt vsiRNAs in each library is shown in parentheses.

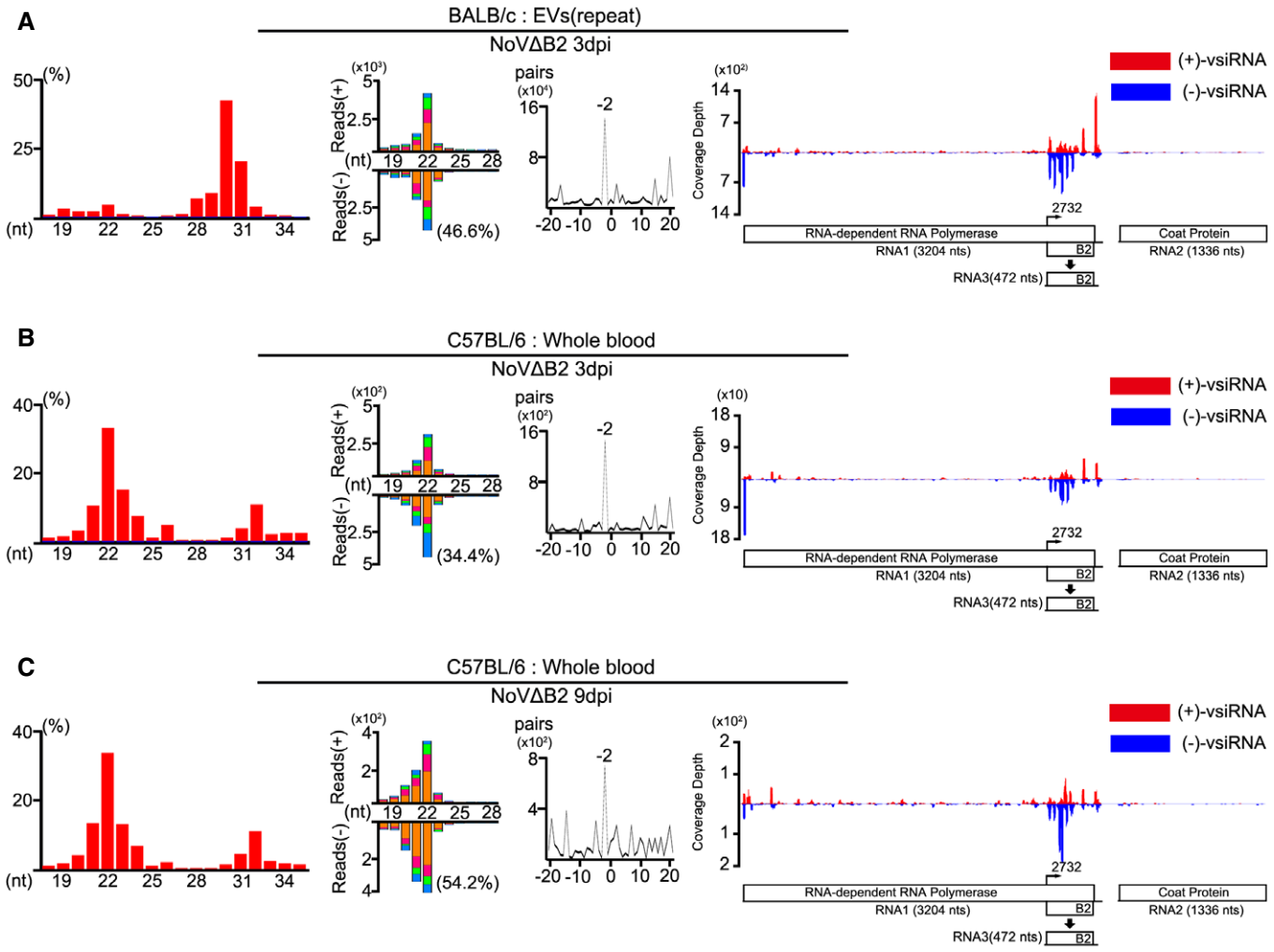

Figure EV2. Properties of total small RNA reads and vsiRNAs.

A–C Properties of total small RNA reads and vsiRNAs sequenced from the EVs in BALB/c suckling mice (A, a repeat of Fig 3), whole blood in C57BL/6 suckling mice infected with NoVΔB2 at 3 dpi (B) and 9 dpi (C). Length distribution of total 18- to 35-nt reads in each library, size distribution of virus-derived siRNAs, duplexes by 22-nt vsiRNAs, and viral genomic coverage depth of each nucleotide position by 21- to 23-nt vsiRNAs are presented as described in previous figures. Reads are shown as per million total mature miRNAs. 5′ terminal nucleotide and 1 U % of 21- to 23-nt vsiRNAs are indicated.

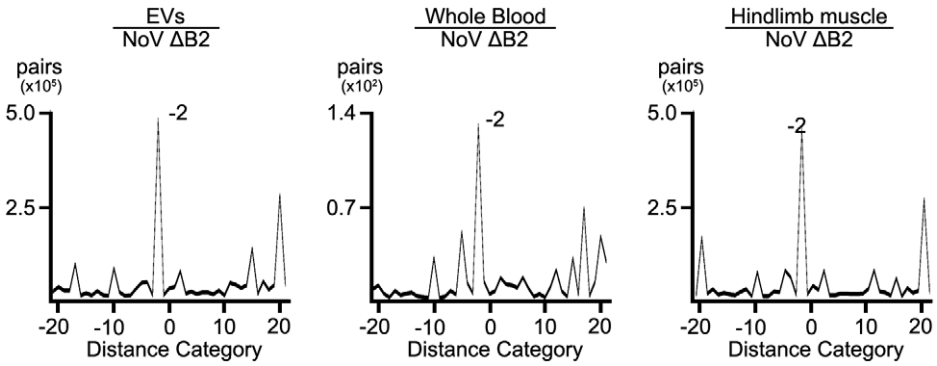

Figure EV3. Duplex pattern of 22-nt vsiRNAs in EVs, whole blood, and hindlimb muscle tissue from NoVΔB2 infected suckling mice.

Peaks at −2 nt and 20 nt indicated a typical duplex model by cleavage of endoribonuclease Dicer.

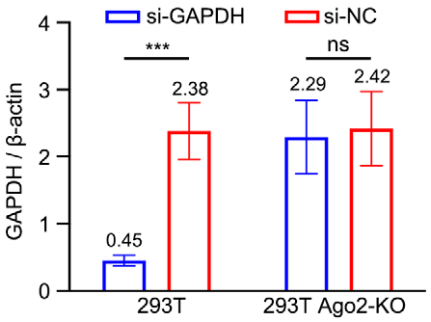

Figure EV4. Functional assay of 293T Ago2-KO cells.

293T and 293T Ago2-KO cells were transfected with siRNAs targeting GAPDH mRNA (si-GAPDH) or control siRNAs (si-NC). The accumulation levels of GAPDH mRNA were quantified by RT–qPCR. Normalization was done by β-actin mRNA. Three biological replicates were performed, and data are shown as mean ± SD. *** indicates $P < 0.001$, ns indicates no significant difference by Student's *t*-test.

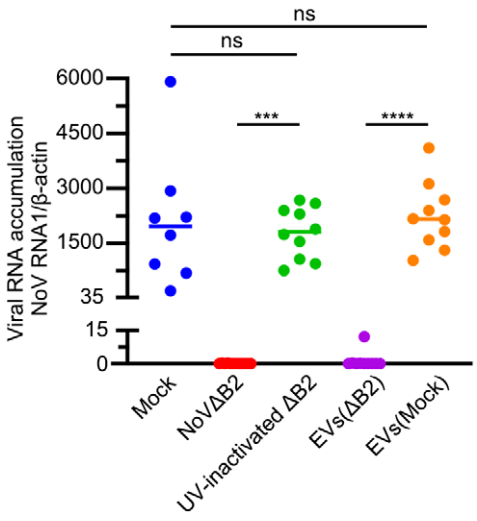

Figure EV5. Long-term protection by NoVΔB2 and EVs (ΔB2).

BABL/c suckling mice were immunized with DMEM, live NoVΔB2, UV-inactivated NoVΔB2, or EVs from mock or NoVΔB2-immunized mice. At 21 days post immunization, the mice were challenged with WT NoV by intraperitoneal injection. These challenged mice were sacrificed at 3 dpi, and NoV RNA1 levels in hindlimb muscle tissue were determined by RT–qPCR. Normalization was done by β-actin mRNA. *** indicates $P < 0.001$, **** indicates $P < 0.0001$, ns indicates no significant difference by Student's *t*-test, $n = 8$–10 per group. Data are shown as mean ± SD.