

Supporting Information for

Live-attenuated virus vaccine defective in RNAi suppression induces rapid protection in neonatal and adult mice lacking mature B and T cells

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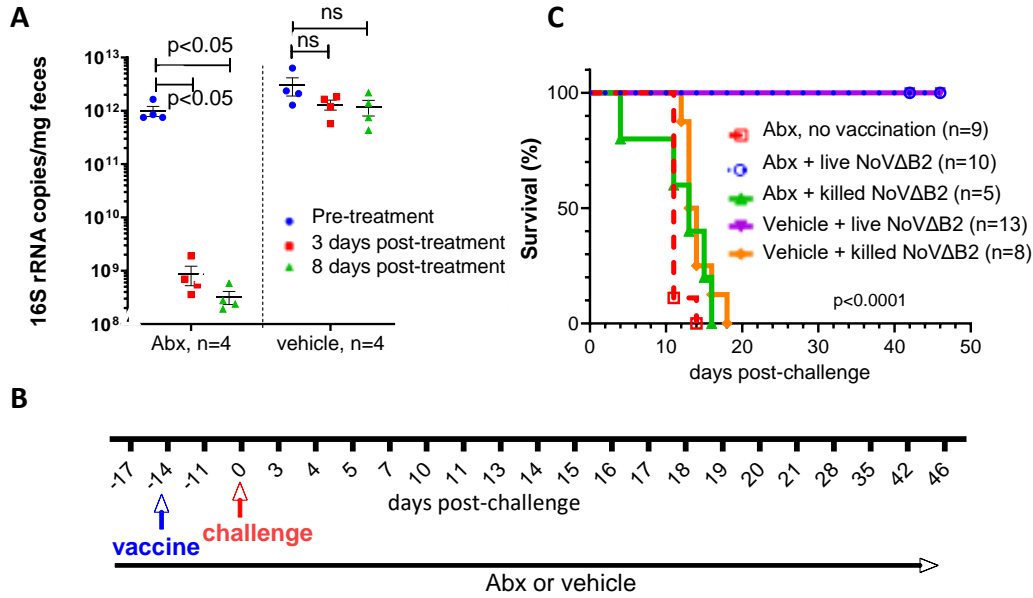


Fig. S1. Immunization with live NoVΔB2 induced efficient protection against NoV challenge in adult *Rag1*^{-/-} mice depleted of the intestinal microbiome. (A) Bacterial 16S rRNA copy number in fecal samples harvested from adult *Rag1*^{-/-} mice prior to the treatment (pre-treatment) or 3 and 8 days after an oral antibiotics cocktail (ampicillin and vancomycin) (Abx) or vehicle (post-treatment) was added in drinking water as described (1). Abx treatment led to significantly diminished fecal bacteria at both time points. ns, not significant. (B) Adult *Rag1*^{-/-} mice were treated with Abx or vehicle in drinking water for 3 days before immunization (and throughout the whole experiment) with live or killed NoVΔB2, and 14 days later, challenged with a lethal dose of NoV. (C) Survival of the adult *Rag1*^{-/-} mice that were vehicle- or Abx-treated before immunization with live or killed NoVΔB2 and 14 days later, challenged with a lethal dose of NoV. Full protection against lethal NoV challenge was induced by immunization with live NoVΔB2 in not only vehicle-treated adult *Rag1*^{-/-} mice, but also Abx-treated, microbiome-depleted adult *Rag1*^{-/-} mice. The data were combined from 3 experiments.

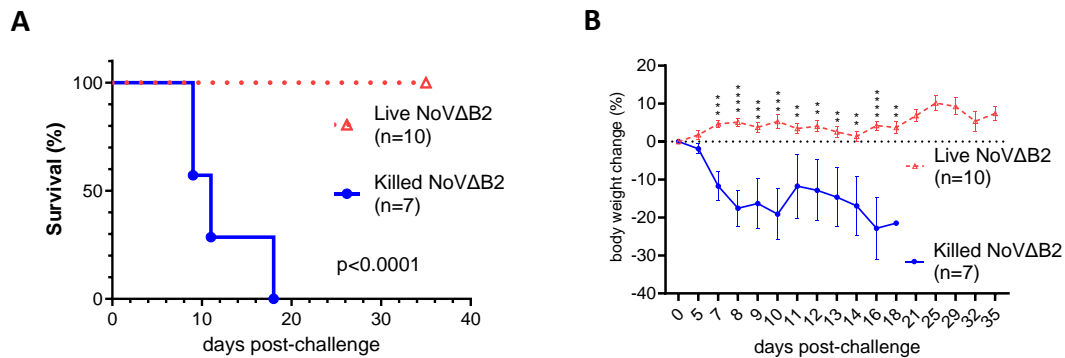


Fig. S2. Rapid induction of protection by the live-attenuated NoVΔB2 in adult *Rag2*^{-/-} *yc*^{-/-} double knockout mice. Survival (A) and body weight change (B) of the adult double knockout mice that were immunized with live or killed NoVΔB2 and 14 days later, challenged with a lethal dose of NoV. Survival curves were compared using a log rank (Mantel-Cox) test whereas body weight changes between immunization by live and killed NoVΔB2 were analyzed by Multiple unpaired t tests (** p<0.01; *** <0.001; **** <0.0001)

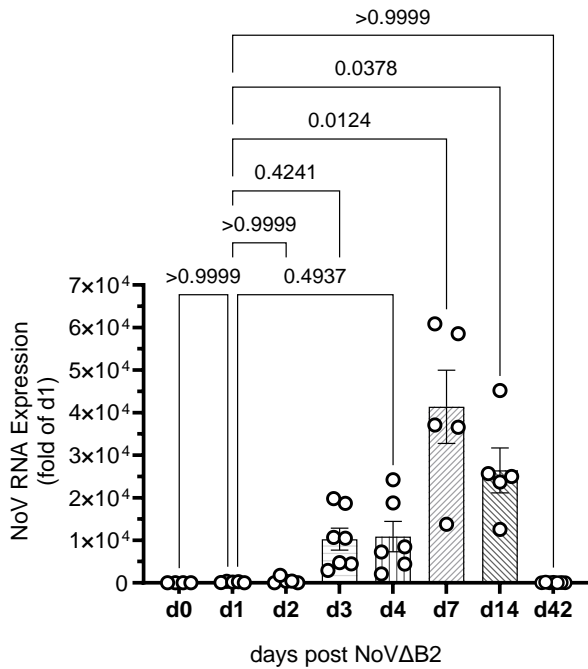


Fig. S3. A time-course analysis of NoVΔB2 accumulation in the immunized *Rag1*^{-/-} adult mice. RT-qPCR analysis of the viral RNA1 accumulation levels using b-actin mRNA as the internal reference was performed on the total RNAs extracted from mouse hind limb skeletal muscle tissues at 1 (n=5), 2 (n=5), 3 (n=7), 4 (n=6), 7 (n=5), 14 (n=5) and 42 (n=7) days post-immunization. Naïve mice (d0, n=4) were used as control. The virus RNA titer in the immunized mice at day 1 was set as 1, data presented are means ± SEM with black circles representing the individual values, and p values indicate the statistical differences compared to that at 1 day post-immunization using One-Way ANOVA.

References

1. E. S. Winkler *et al.*, The Intestinal Microbiome Restricts Alphavirus Infection and Dissemination through a Bile Acid-Type I IFN Signaling Axis. *Cell* **182**, 901-918 e918 (2020).