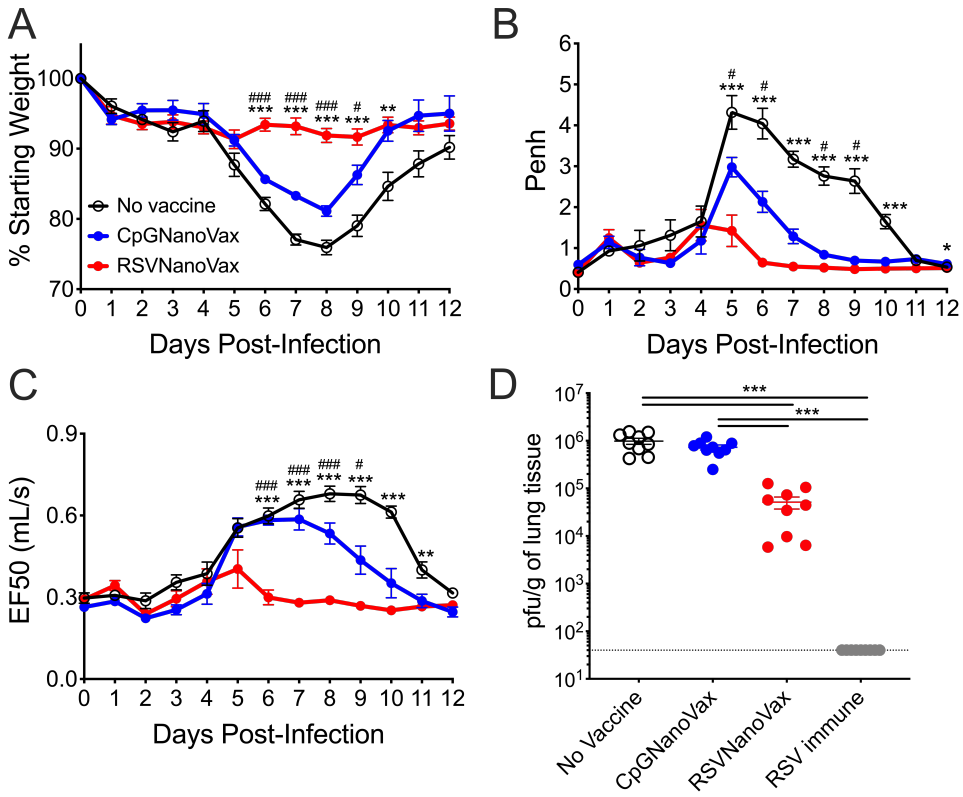

Figure S1. Single dose of RSVNanoVax reduces RSV-induced disease but fails to mediate viral clearance. BALB/c mice were primed with 500 μg of the indicated nanoparticle formulation i.n. on day 0, and challenged with 4.8 x 10^6 PFU RSV-A2 i.n. on day 28. All mice were assessed daily for **(A)** weight loss, **(B)** Penh, and **(C)** EF50. **(D)** Infectious viral PFU were quantified in the lung on day 4 post-infection by plaque assay. (A-C) Asterisks represent significance between no vaccine and RSVNanoVax and pound symbols represent significance between CpGNanoVax and RSVNanoVax as determined by 2-way ANOVA with Dunnett's post hoc test. (D) Statistical significance was determined by one-way ANOVA with a a Tukey's post hoc test. $^{*/\#}$ p<0.05, $^{**/\#\#}$ p<0.01, $^{***/\#\#\#}$ p<0.001. No vaccine mice were administered PBS i.n. and RSV immune mice received 4.8 x 10^6 PFU RSV-A2. Data represent mean±SEM of 2 independent experiments (n=8-9). The horizontal dashed line represents the limit of detection.

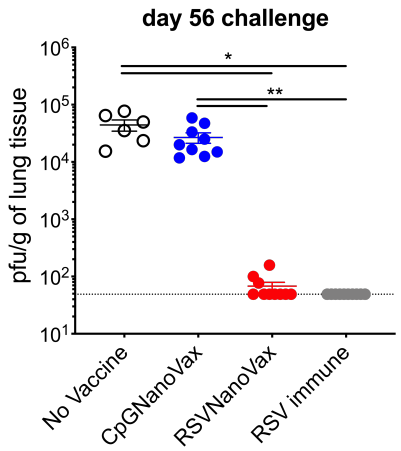

Figure S2. Prime-boost RSVNanoVax immunization reduces infectious RSV particles in the lungs on day 2 post-infection. BALB/c mice were primed i.n. on day 0 and boosted with 500 μg on day 28. On day 56, mice were challenged with 4.8 x 10^6 PFU RSV-A2 and infectious viral pfu were quantified in the lung on day 2 post-infection. Statistical significance was determined by one-way ANOVA with a Tukey's post hoc test. $^{*}p<0.05$, $^{**}p<0.01$. No vaccine mice were administered PBS i.n. at both the prime and boost. RSV immune mice received 4.8 x 10^6 PFU RSV-A2 at the prime and PBS i.n. at the boost. Data represent mean±SEM of 2 independent experiments (n=7-10). The horizontal dashed line represents the limit of detection.

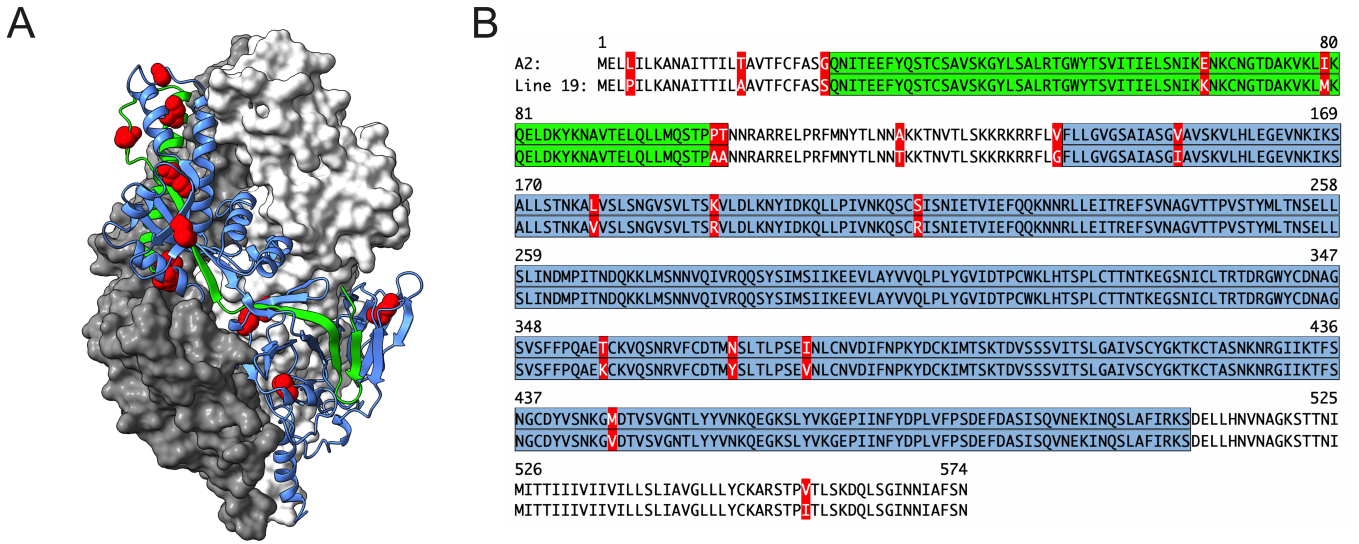

Figure S3. Amino acid sequences of the RSV-A2 and RSV line 19 F proteins. (A) 3D protein structure of the RSV fusion protein in the prefusion conformation. Two chains of the homotrimer are depicted as molecular surfaces and colored gray and white. The final chain is shown as a ribbon cartoon. The F_1 and F_2 subunits are colored blue and green, respectively. Residues which differ between RSV A2 and line 19 are shown as spheres colored red. **(B)** Amino acid sequence with substitution differences between A2 and line 19 highlighted in red. The F_2 region of the protein is highlighted in green and the F_1 region of the protein is highlighted in blue.