Supplementary Materials

Table S1: RT-qPCR viral load results following the transfection of pSinCCHF-52S in BHK-21 cells. Samples were analyzed in duplicate.

Time after electroporation	**Ct value**	**Estimated RNA copy number**	**Average RNA copy number**
4 hrs 4 hrs	28.52 28.68	*NE	*NE
8 hrs 8 hrs	24.31 24.21	4.09 E3 4.31 E3	4.23 E3
12 hrs 12 hrs	22.86 23.01	1.06 E4 9.6 E3	9.65 E3
24 hrs 24 hrs	22.01 21.96	1.85 E4 1.91 E4	1.88 E4
48 hrs 48 hrs	22.98 23.16	9.78 E3 8.7 E3	9.24 E3

*NE – CCHFV RNA copy numbers not estimated

Table S2: Spearman Rank Order Correlations. MD pairwise deleted. Correlations in bold are significant at $p < 0.001$

Variable	**Anti-52S IgG1**	**Anti-52S IgG2a**	**Anti-52S IgG2b**	**Anti-52S IgG2a/IgG1**	**Anti-52S IgG2a/IgG2b**	**IFN-γ**	**IL-2**	**TNF-α**	**IL-10**	**IL-6**
Anti-52S IgG1	1.000000	**0.927173**	**0.993399**	-0.750000	-0.883883	0.906539	0.906539	**0.974679**	0.468692	**0.951190**
Anti-52S IgG2a	**0.927173**	1.000000	**0.933333**	0.000000	-0.304290	**0.950586**	**0.950586**	**0.968246**	0.363419	0.818923
Anti-52S IgG2b	**0.993399**	**0.933333**	1.000000	-0.645497	-0.912871	0.887214	0.887214	**0.968246**	0.446304	**0.944911**
Anti-52S IgG2a/IgG1	-0.750000	0.000000	-0.645497	1.000000	0.824958	-0.223607	-0.223607	-0.447214	-0.223607	-0.894427
Anti-52S IgG2a/IgG2b	-0.883883	-0.304290	-0.912871	0.824958	1.000000	-0.263523	-0.263523	-0.632456	0.263523	-0.948683
IFN-γ	0.906539	**0.950586**	0.887214	-0.223607	-0.263523	1.000000	0.921687	**0.932673**	0.448493	0.790433
IL-2	0.906539	**0.950586**	0.887214	-0.223607	-0.263523	0.921687	1.000000	**0.957217**	0.375764	0.778457
TNF-α	**0.974679**	**0.968246**	**0.968246**	-0.447214	-0.632456	**0.932673**	**0.957217**	1.000000	0.395092	0.902708
IL-10	0.468692	0.363419	0.446304	-0.223607	0.263523	0.448493	0.375764	0.395092	1.000000	0.542208
IL-6	**0.951190**	0.818923	**0.944911**	-0.894427	-0.948683	0.790433	0.778457	0.902708	0.542208	1.000000

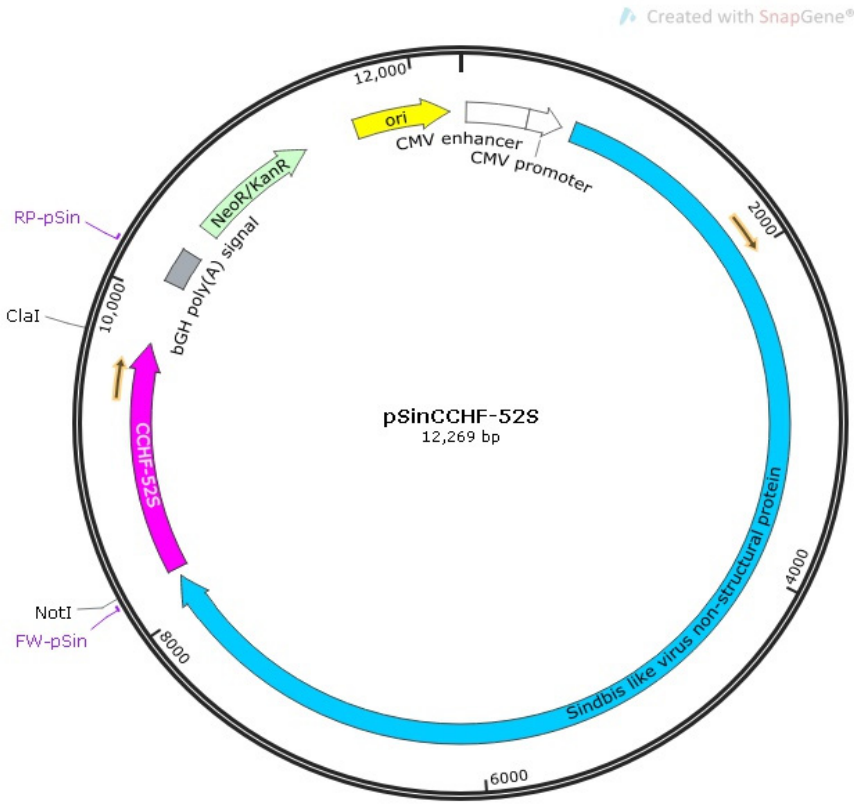

Figure S1. Preparation of recombinant plasmids. Schematic representation of pSinCCHF-52S.

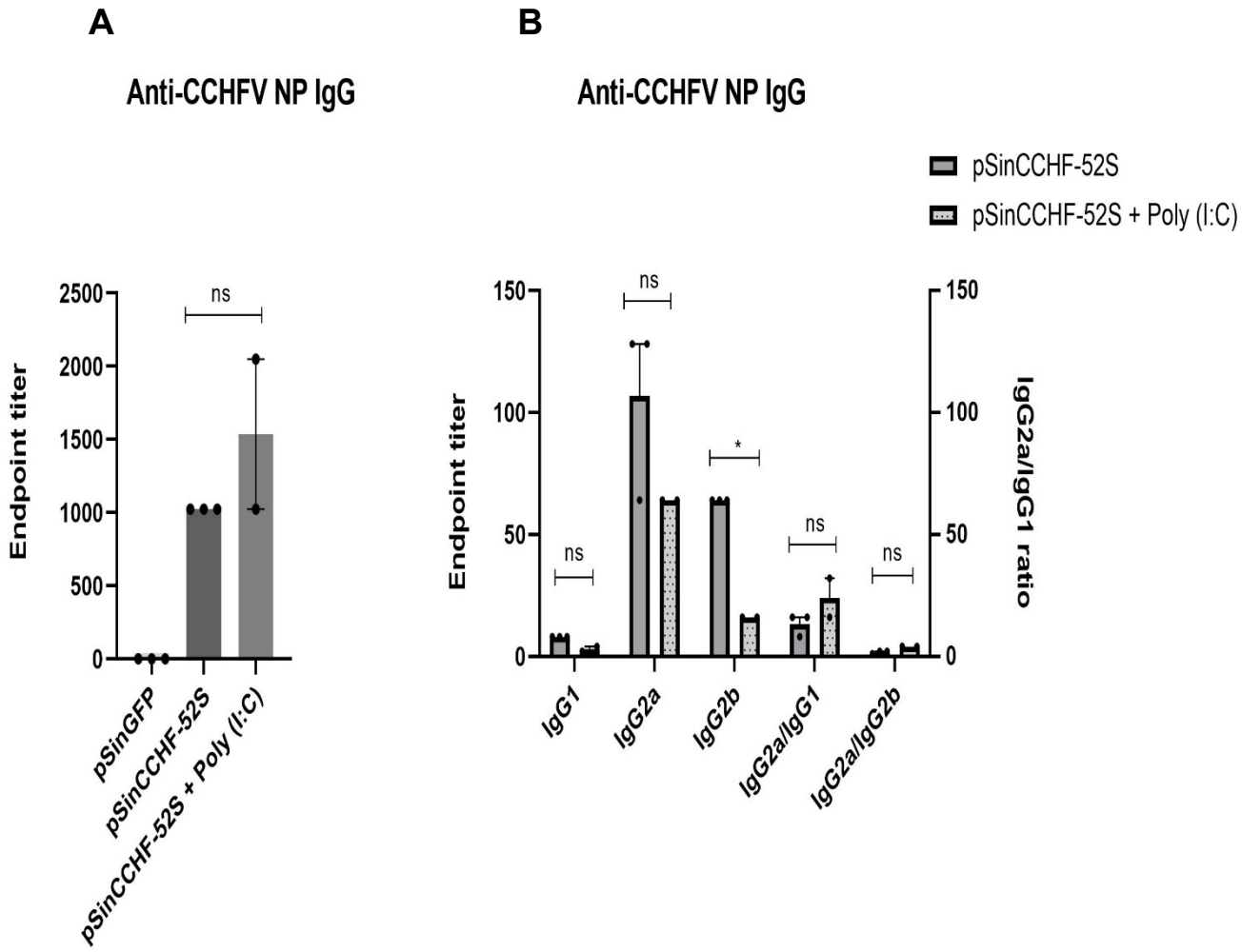

Figure S2. Antibody responses induced by immunization with pSinCCHF-52S with and without Poly (I:C). **(A)** Total anti-CCHFV NP endpoint titer **(B)** Anti-CCHFV NP IgG isotypes endpoint titer. Mice (NIH; n = 3/group) were immunized three times intramuscularly with the prepared pSinCCHF-52S construct expressing CCHFV nucleoprotein with and without Poly (I:C). Serum anti-CCHFV NP IgG subtypes were analyzed using an indirect immunofluorescent assay. Data are expressed as the mean for three mice (pSinCCHF-52S) and two mice for pSinCCHF-52S+Poly (I:C) and the standard error of the mean. * Statistical analysis performed using the F test.

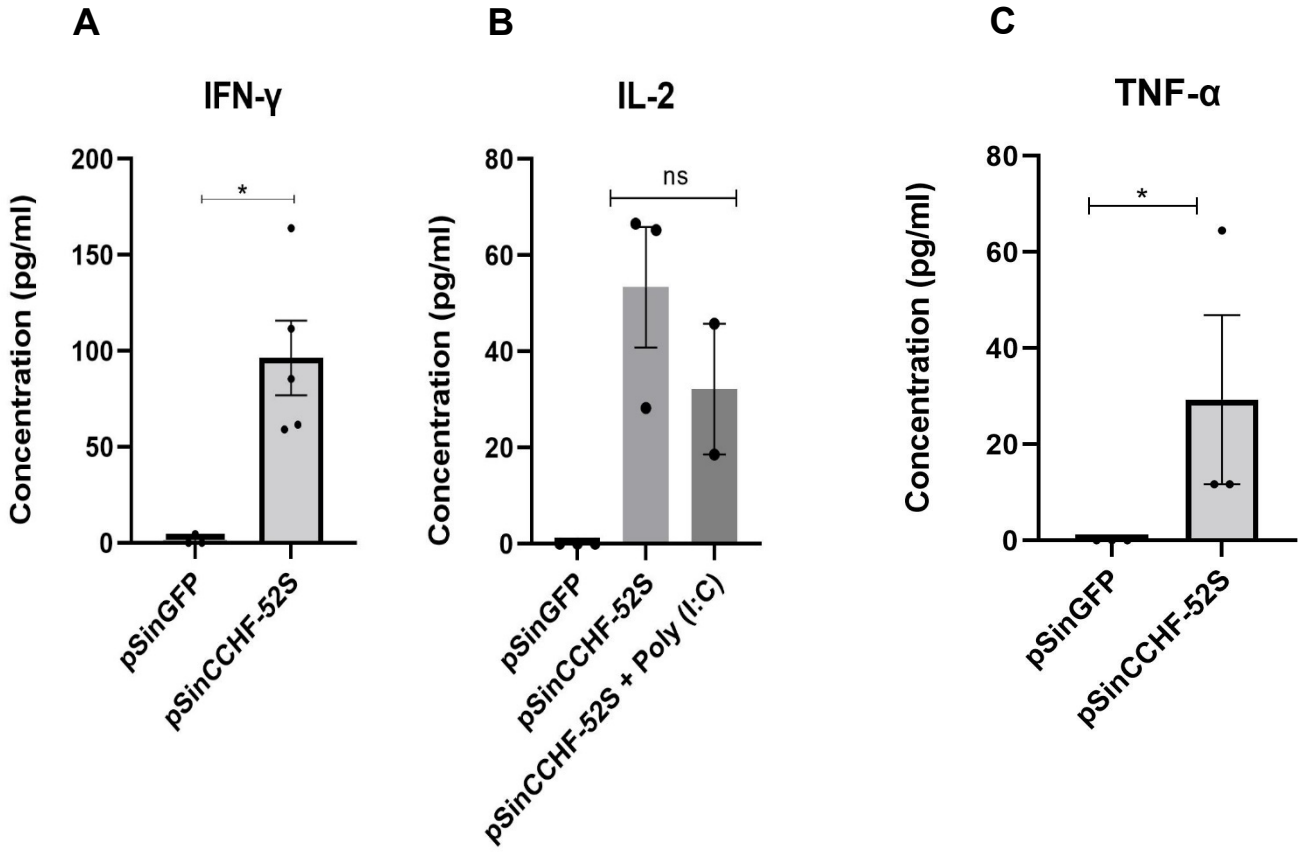

Figure S3. Cytokine profiling by ELISA from splenocytes harvested from NIH mice after immunization with pSinCCHF-52S independently (n = 3) or with adjuvant poly (I:C) (n = 2). (**A**) IFN-γ, (**B**) IL-2, (**C**) TNF-α. Data are expressed as the mean for three mice (except for pSinCCHF-52S + Poly (I:C), n = 2/group) and the standard error of the mean. * $p < 0.05$.

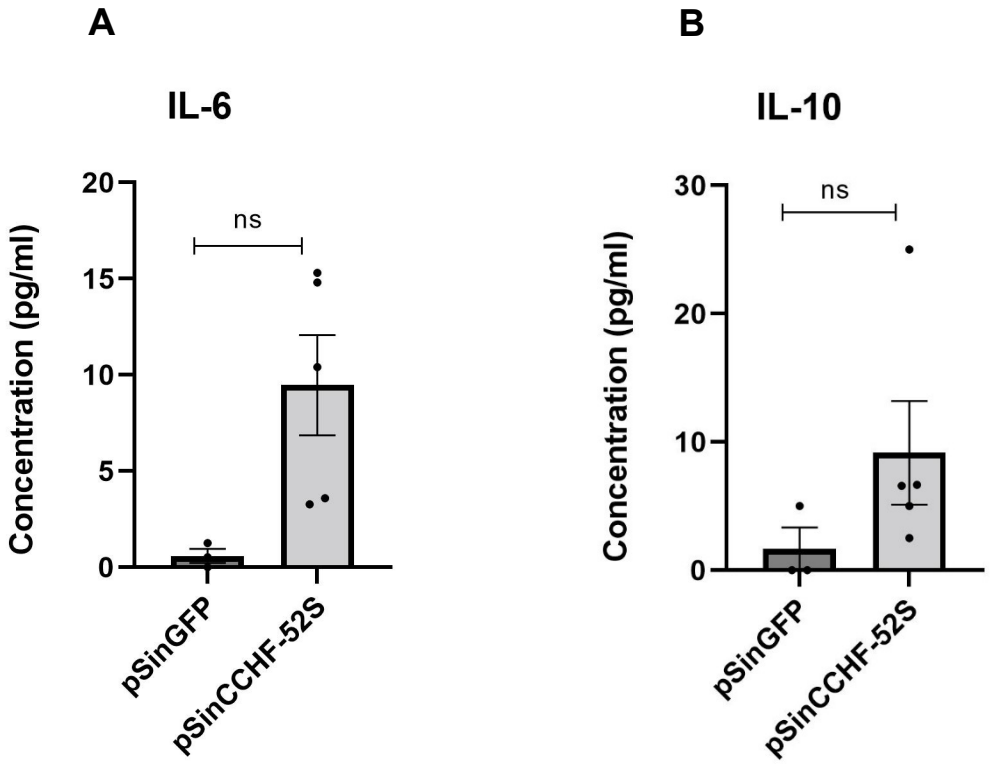

Figure S4. Cytokine profiling by ELISA from splenocytes harvested from NIH mice (n = 5/group) after immunization with pSinCCHF-52S independently or with adjuvant poly (I:C). (**A**) IL-6, (**B**) IL-10. Data are expressed as the mean for three mice (except for pSinGFP, n = 3/group) and the standard error of the mean. * $p < 0.05$.

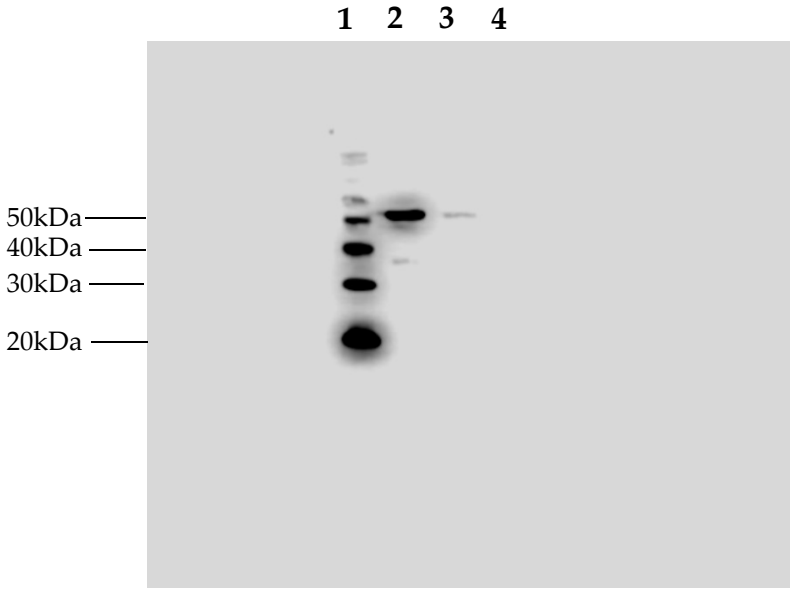

Figure S5: Western blot analysis of CCHFV NP. From left to right; Lane 1: MagicMark XP Western Protein Standard, lane 2 BHK-21 cells transfected with pSinCCHF-52S, Lane 3 BHK-21 cells transfected with pSinCCHF-31S (Replicon kinetics and immunogenicity data not reported in this study), Lane 4 Mock-transfected BHK-21 cells.