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Supplemental Information

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Self-amplifying RNA vaccine protects mice against lethal Ebola virus infection

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Supplemental Figure 1

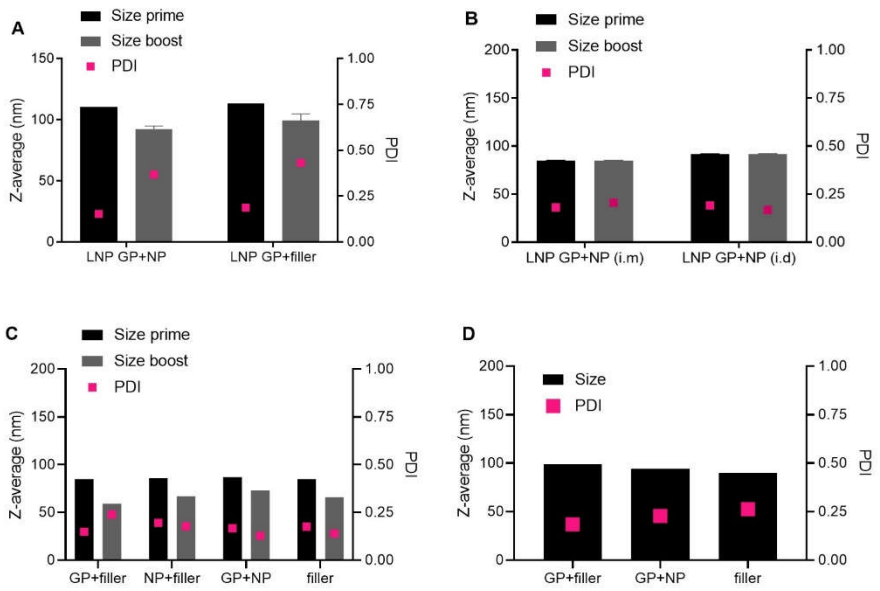

Figure S1. Hydrodinamic diameter (Z-Average) and polydispersity index (PDI) of LNPs used in the experiments by dynamic Light Scattering (DLS). (A) LNPs co-formulated with GP saRNA/NP saRNA or GP saRNA/filler RNA showed identical hydrodynamic diameter and PDI, either in the prime injection or boost injection represented in Figure 2. A measurable increment of PDI between both formulations used for the boost is accompanied by an overall reduction in the hydrodynamic diameter. (B) LNPs co-formulated with GP saRNA/NP saRNA either for i.m or i.d application, depicted in Figure 3, showed identical hydrodynamic diameter and PDI. (C) No differences could be observed in the hydrodynamic diameter or PDI of the LNPs after co-formulation of GP saRNA/filler RNA or NP saRNA/filler RNA or GP saRNA/NP saRNA or filler alone, that were used for the prime or boost injection in the challenge experiment depicted in Figure 4. (D) LNPs co-formulated with either GP-saRNA/filler or GP-saRNA/NP-saRNA or filler for the challenge experiment in Figure 5, showed identical hydrodynamic diameter and PDI.

29 **Supplemental Table 1: Evaluation table of clinical scores during challenge experiments**

Scoring	0	1	5	10
Body weight (deviation from average individual weight*)	0 % – 5 %	6 % – 10 %	11 % – 15 %	> 15 %
General condition	Fur smooth, shiny; Orifices clean; Eyes clear; no deviation in body temperature	Fur slightly ruffled, poor or excessive grooming; no deviation in body temperature	Fur dull, disordered; unkempt orifices; Eyes cloudy; Deviation of the body temperature up to 1 ° C	Focally sticky or shaggy, rough fur; Eyes cloudy; Diarrhea; Jaundice or evidence of coagulopathy; Deviation of the body temperature up to 2 ° C
Spontaneous behaviour	Normal behaviour (sleeping, reacting to touch, curiosity, social contact)	Minor deviations from normal behaviors	Moderate changes in activity (hyperactivity / hypoactivity)	Severe activity changes or lethargy; Self-isolation, aggressive behavior towards oneself or cage mates

*The average individual body weight was determined by measuring on three different days before the experiment.

The score in the three different categories (body weight, general condition and spontaneous behaviour) for each animal is summed up and animals were euthanized when they reached a score of 10, i.e. the evaluation in one of the criteria body weight, general condition and spontaneous behaviour with a score of 10 was sufficient for euthanasia. If, by adding up the results of the various criteria, a score of 10 on a single day or 6 on two consecutive days was obtained, the veterinarian was consulted to decide on the euthanasia.

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Supplemental Table 2: Peptides used in ELISpot assays

Peptide name	Amino acid sequence
Peptides derived from EBOV GP	
MHC class I	
GP MHC-I 1	FEVDNLTYV
GP MHC-I 2	MASENSSAM
GP MHC-I 3	FSILNRKAI
GP MHC-I 4	DNLTYVQL
GP MHC-I 5	AFFLYDRL
GP MHC-I 6	ELRTFSIL
MHC class II	
GP MHC-II 1	GLAWIPYFGPAAEGI
GP MHC-II 2	KRWGFRSGVPPKVVN
Peptides derived from EBOV NP	
MHC class I	
NP MHC-I 1	YQVNNLEEI
NP MHC-I 2	ARLLNLSGV
NP MHC-I 3	DGVKRLEEL
MHC class II	
NP MHC-II 1	EVNSFAKALSSLAKH
NP MHC-II 2	TSNRTTPVAPPAPVY