Supplementary Information

Manuscript title: A Pilot Study on a Heterologous Prime-Boost Approach for Inducing an Immune Response against Double-Layered Human Rotavirus Particles

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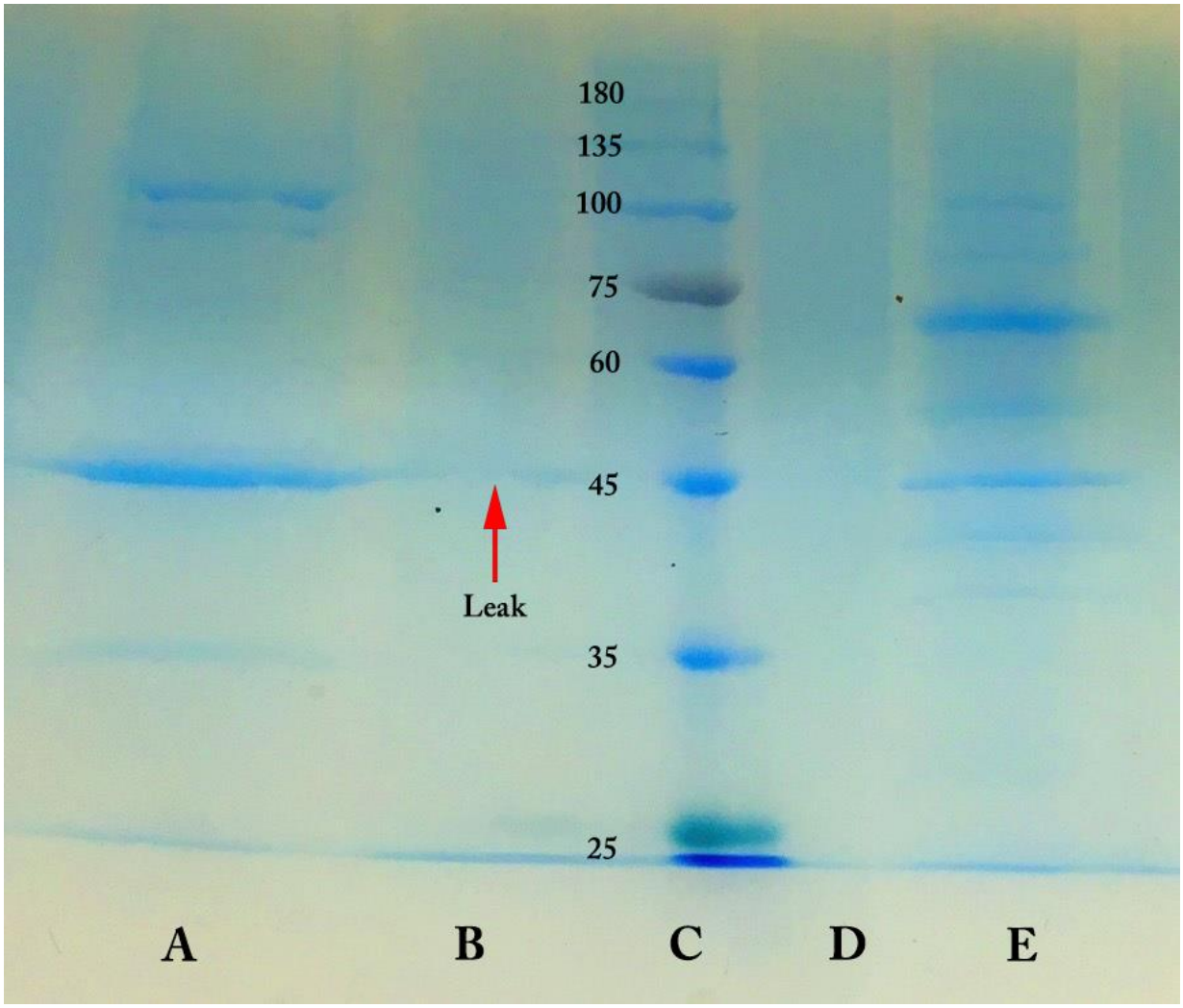
Gel/Blot Original Images



Supplementary 1. Full-Length SDS-PAGE Gel for Fig. 3a

This image displays the full, unprocessed SDS-PAGE gel used to generate the cropped image in Fig. 3a of the manuscript. The gel shows an SDS-PAGE performed to compare the quality of rotavirus group A (RVA) structural proteins before and after the dialysis of purified RVAs against TNC buffer. Some minor weight discrepancies might be due to glycosylation or potential degradation, which can alter the final pattern observed on the gel. This image was cropped, keeping lanes C, D, and E, to be used in the manuscript as Fig. 3a. Lane labels are as follows:

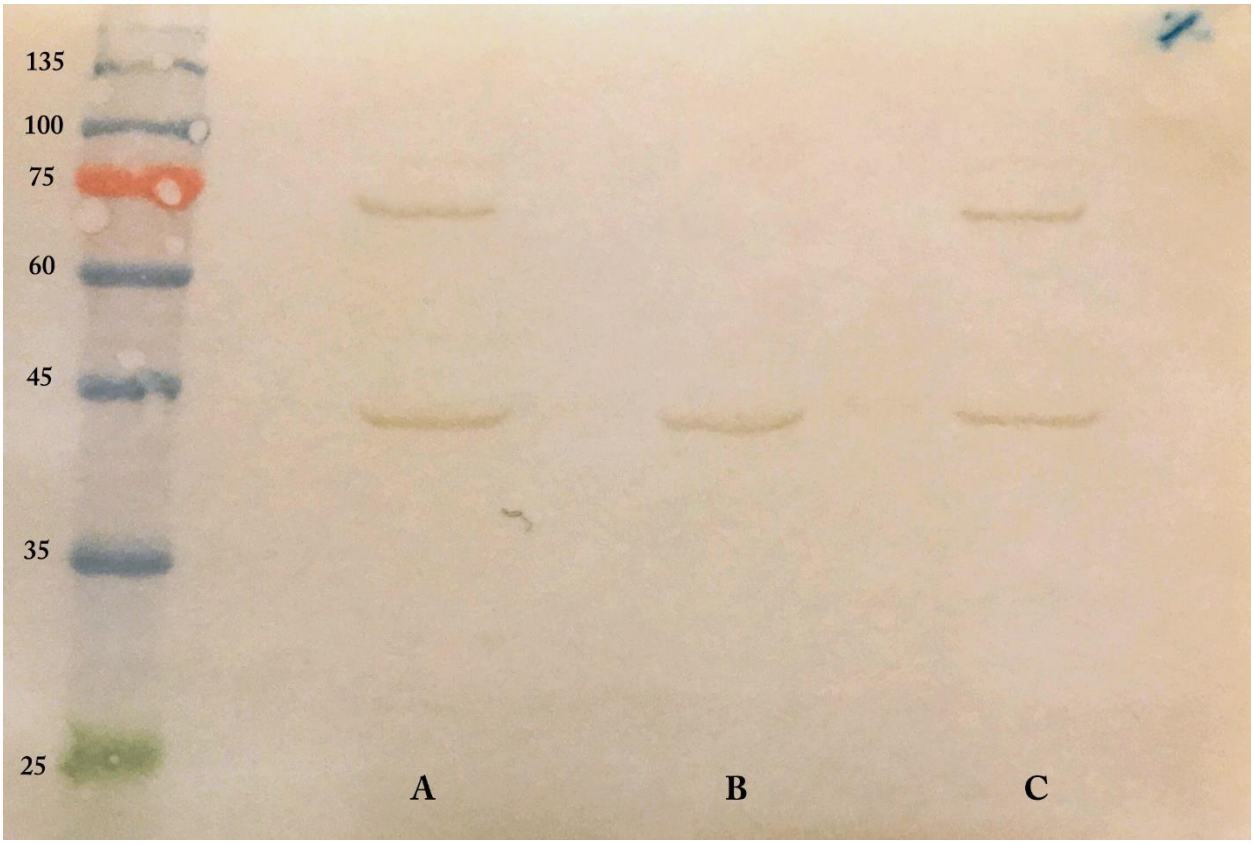
(A) Undialyzed RVA proteins aligned based on their molecular weights

(B) Undialyzed mock-infected cell lysate with no protein bands

(C) Protein size marker

(D) Mock-infected cell lysate after dialysis showing no protein bands

(E) Dialyzed RVA proteins revealing sharper protein bands



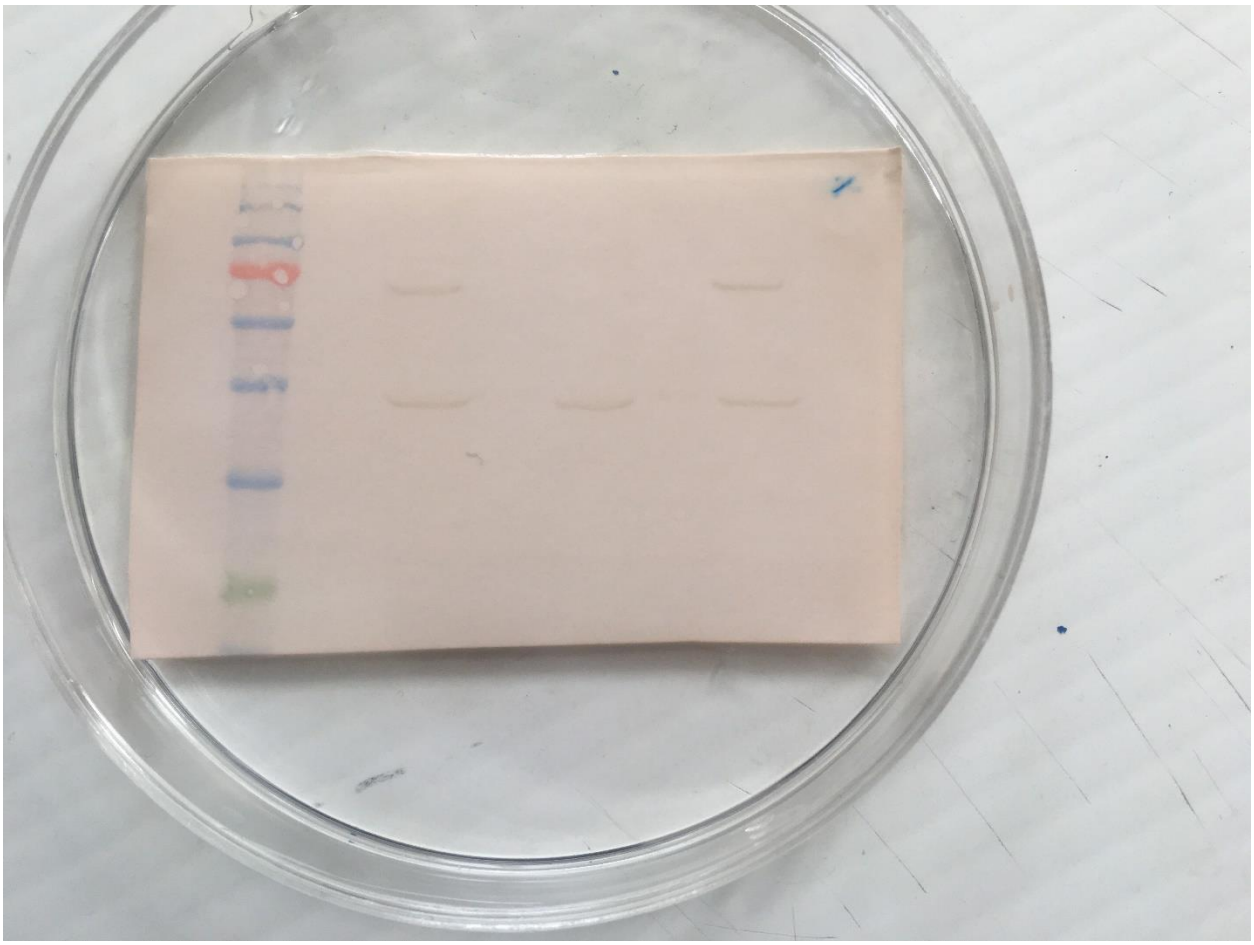
Supplementary 2. Full-Length Western Blot for Fig. 3b

This image presents the full, unprocessed Western blot used for the polished image in Fig. 3b of the manuscript. The blot was probed for VP6 proteins (~44 kDa). Lanes A, B, and C were physically separated using a multichannel incubation tray and exposed to individual rabbit sera simultaneously for western blotting. Lane labels are as follows:

(A) Post-booster rabbit sera of the first group showing VP6 (~44 kDa) and VP4 (~84 kDa) bands

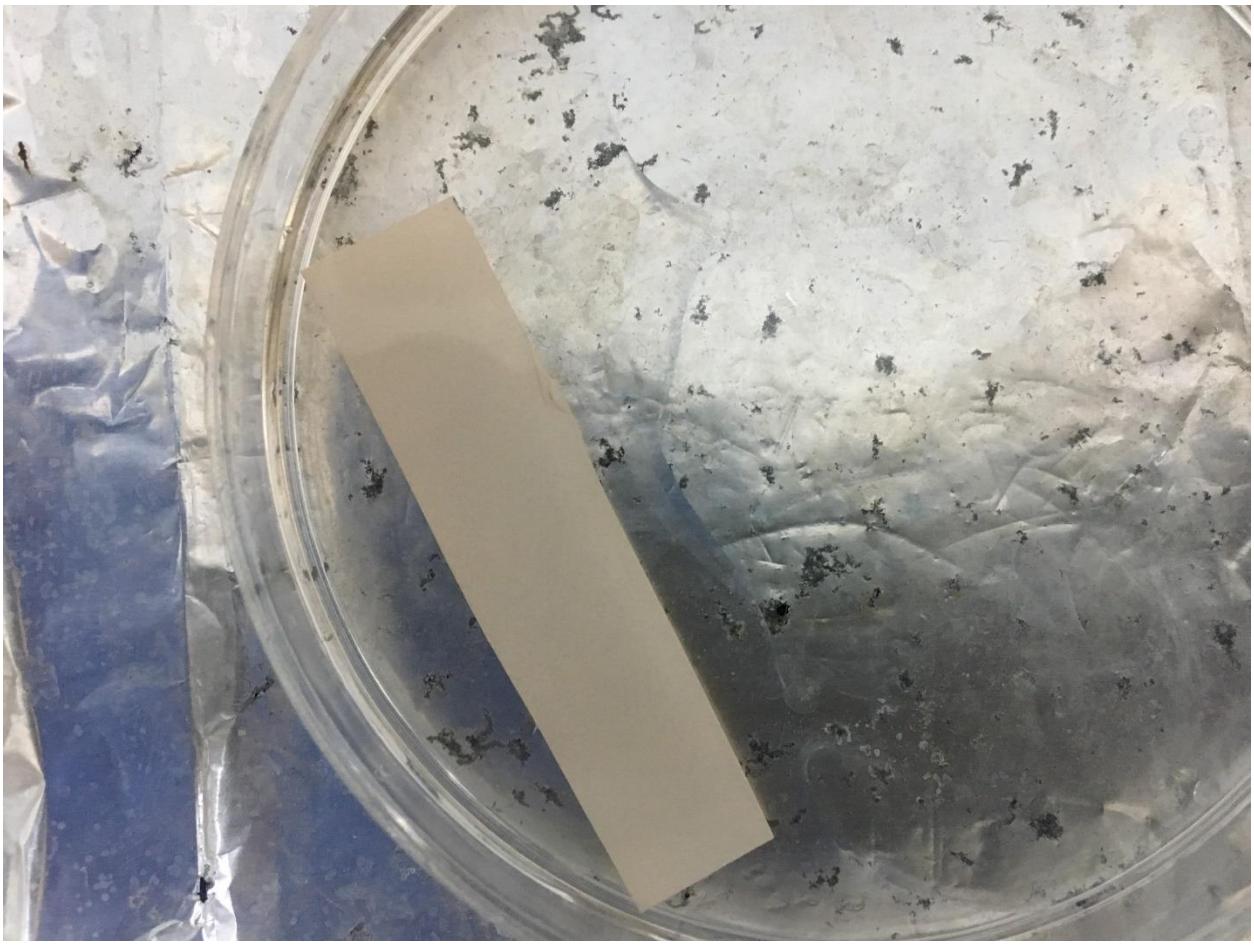
(B) Post-booster rabbit sera of the second group showing only a VP6 (~44 kDa) band

(C) Post-booster rabbit sera of the third group showing VP6 (~44 kDa) and VP4 (~84 kDa) bands



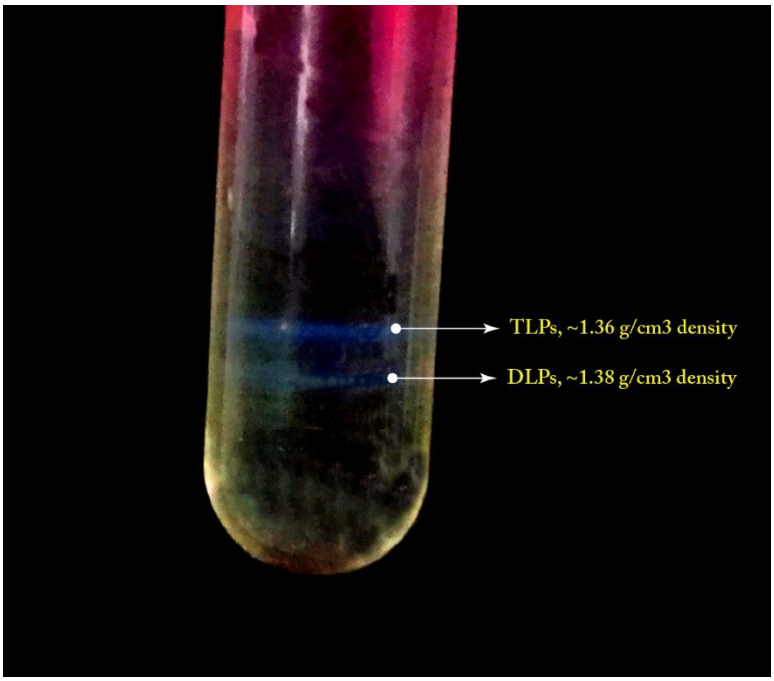
Supplementary 3. Uncropped Western Blot for Fig. 3b

This is the uncropped, unlabeled image in “Supplementary 2” for further review.



Supplementary 4. Uncropped Western Blot of the Negative Control for Fig. 3b

This image shows a strip split from the end of the nitrocellulose membrane, shown in "Supplementary 2" after protein transfer. It was exposed to the post-booster rabbit sera of the fourth group (negative control) simultaneously as the rabbit sera of the first, second, and third groups (lanes A, B, and C in Supplementary 2) were subjected to western blotting. No bands were revealed on "Supplementary 4", and it was later combined into "Supplementary 2" as lane D for Fig. 3b.



Supplementary 5. Purification of RVA by CsCl gradient ultracentrifugation. The figure shows the two distinct bands formed after ultracentrifugation. The upper band consists of TLPs with a density of ~1.36 g/cm^3, and the lower band contains DLPs with a density of ~1.38 g/cm^3.