An Advax-adjuvanted inactivated cell-culture Japanese encephalitis vaccine induces broadly neutralising anti-flavivirus antibodies and T-cell immunity and provides single dose protection

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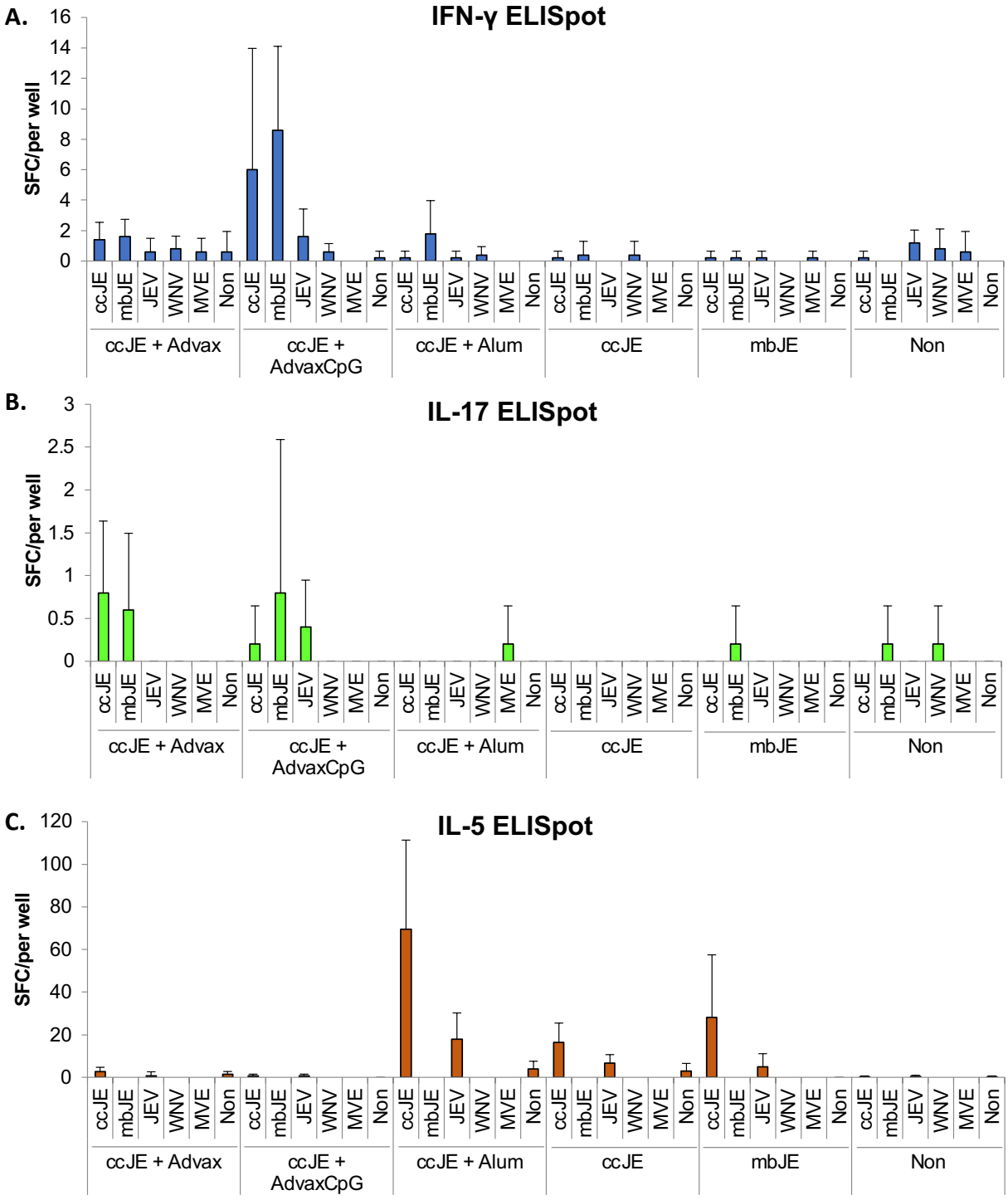
Supplementary Materials

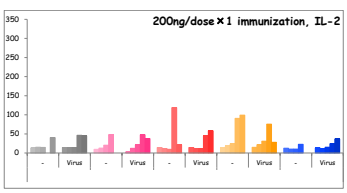
	Challenge Virus				
Immunised Mouse Sera	**JEV**	**WNV**	**MVE**	**DENV1**	**DENV2**
(i) ccJE + Advax	2.798	1.177	1.594	1.420	1.894
(ii) ccJE + AdvaxCpG	2.665	1.203	1.871	1.207	1.907
(iii) ccJE + CpG	2.885	0.445	1.410	0.418	0.894
(iv) ccJE + Alum	2.985	0.961	1.452	0.887	1.805
(v) ccJE	2.889	0.865	1.451	1.020	1.559
(vi) mbJE	3.370	N.D.	1.192	N.D.	0.741

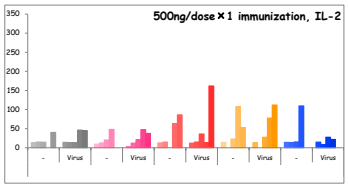
Supplementary Table 1: Addition of CpG confer Advax induces broad cross-neutralising antibodies against Japanese encephalitis virus (JEV) and other Flaviviruses. C57BL/6 mice were immunised and boosted after 3 weeks with mbJE alone and ccJE alone or with Advax, CpG, AdvaxCpG or Alum. Blood was collected 3 week post last immunisation to assess neutralisation activity. Other Flaviviruses include West Nile Virus (WNV), Murray Valley encephalitis virus (MVEV) and Dengue virus 1 and 2 (DENV1/2). Neutralisation titres are presented as log10. Data shown represents pooled sera samples.

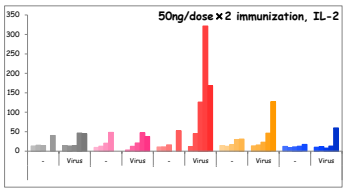


Supplementary Figure 1: Advax with CpG induces strong IFN-γ cellular response. Four-week-old C57BL/6 mice were immunised intramuscularly with mbJE or ccJE alone or with CpG or AdvaxCpG or Alum twice 3 weeks apart with a vaccine antigen dose of 50ng. Spleens were collected 3 weeks after the last immunisation. Antigen-specific **(A)** IFN-γ, **(B)** IL-17 and **(C)** IL-5 producing splenocytes evaluated by ELISPOT following restimulation with 50ng of ccJE or mbJE vaccine, or with JEV, MVE or WNV (MOI=0.01).

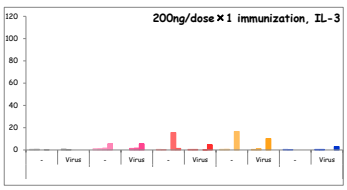
A. IL-2

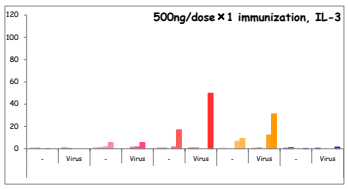


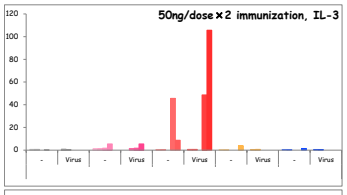




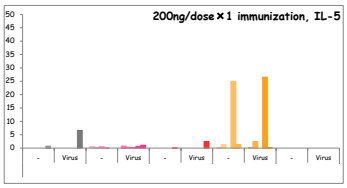
B. IL-3

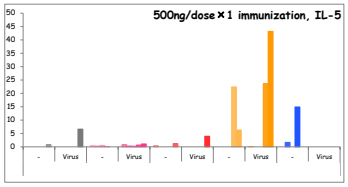


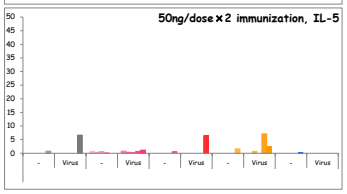




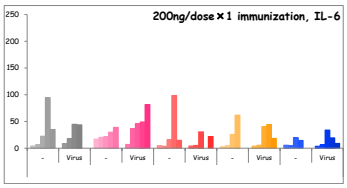
C. IL-5

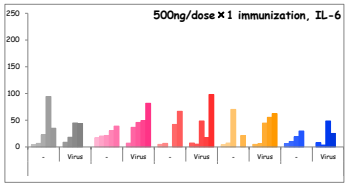


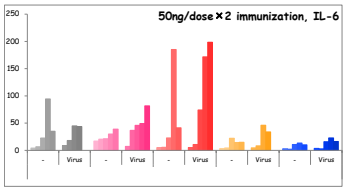




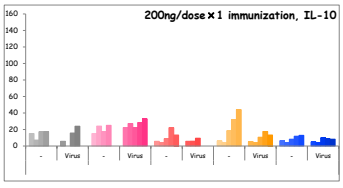
D. IL-6

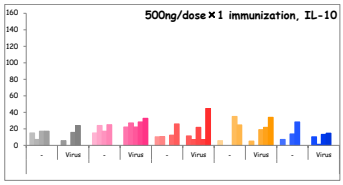


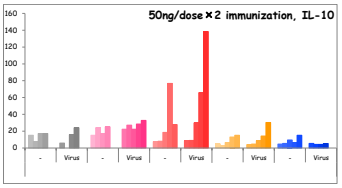




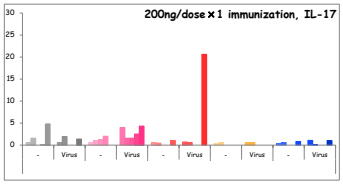
E. IL-10

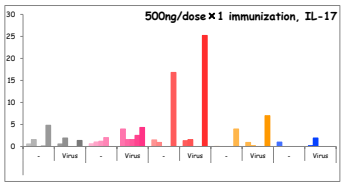


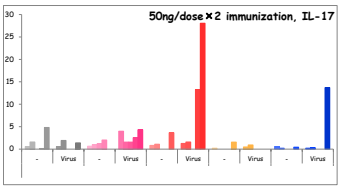




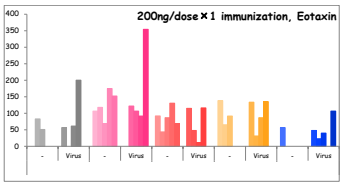
F. IL-17

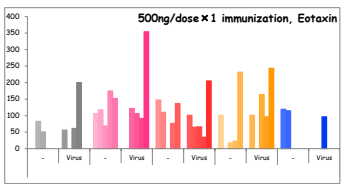


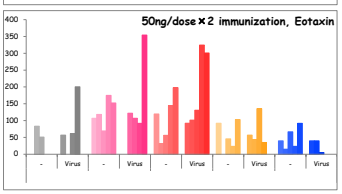




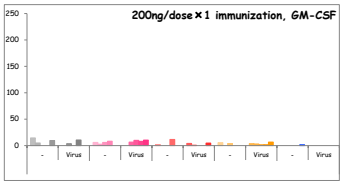
G. Eotaxin

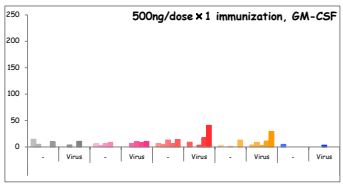


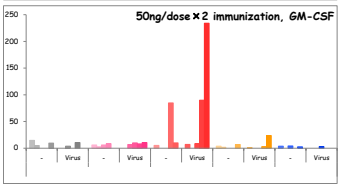


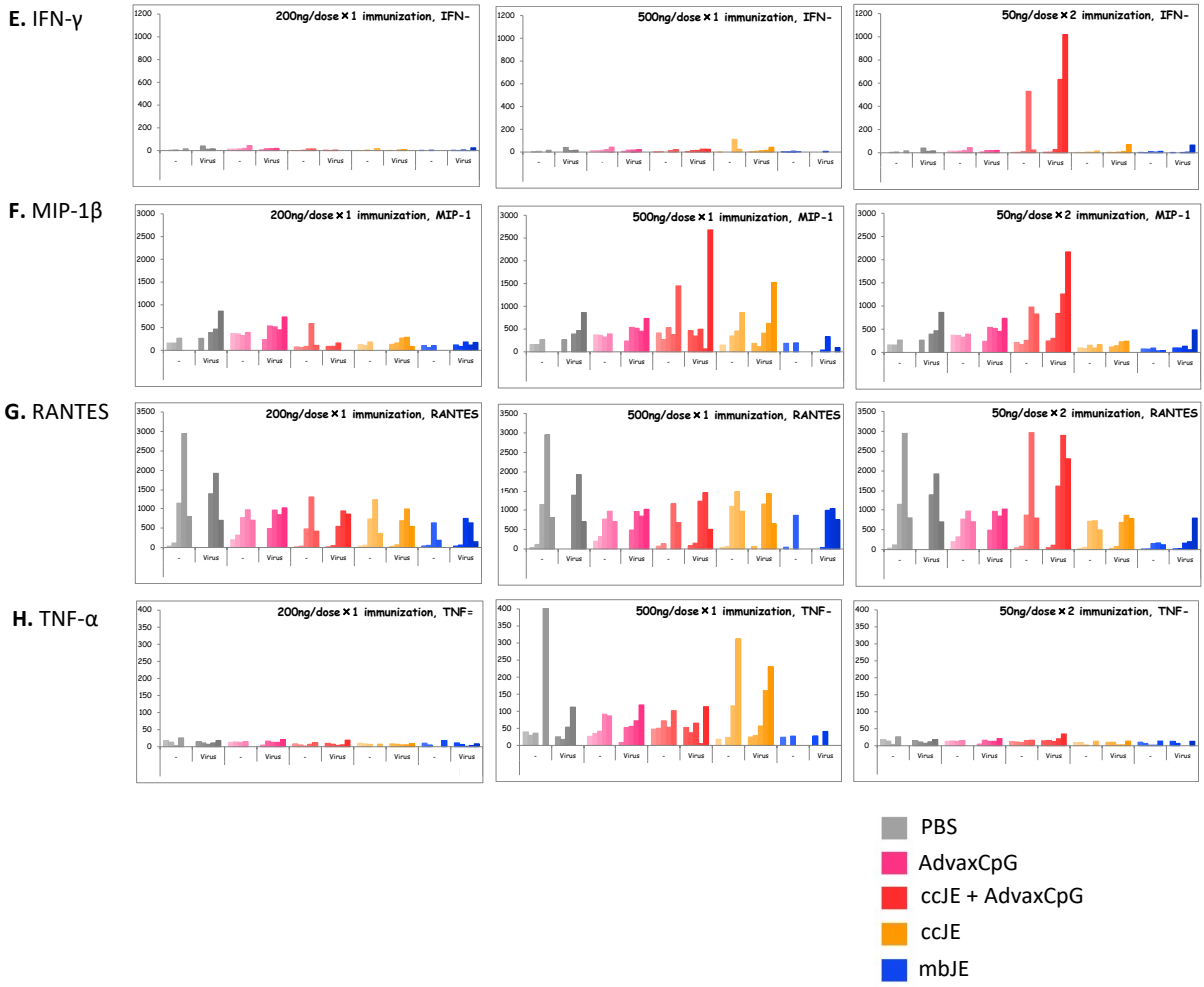


H. GM-CSF









Supplementary Figure 2: Cytokine production T-cells in single and two dose ccJE immunised mice AdavaxCpG. Four-week-old C57BL/6 strain mice were immunised intramuscularly with ccJE alone or with CpG or Advax or AdvaxCpG twice 3 weeks apart with a vaccine antigen dose of 50ng or once with a vaccine antigen dose of 500ng or 200ng. As a control mice were also immunised mbJE or PBS. Three weeks after last immunisation splenocyltes were isolated restimulated with or without virus. Cytokine production was measured using Bio-Plex Mouse Cytokine 23-plex Assay.