

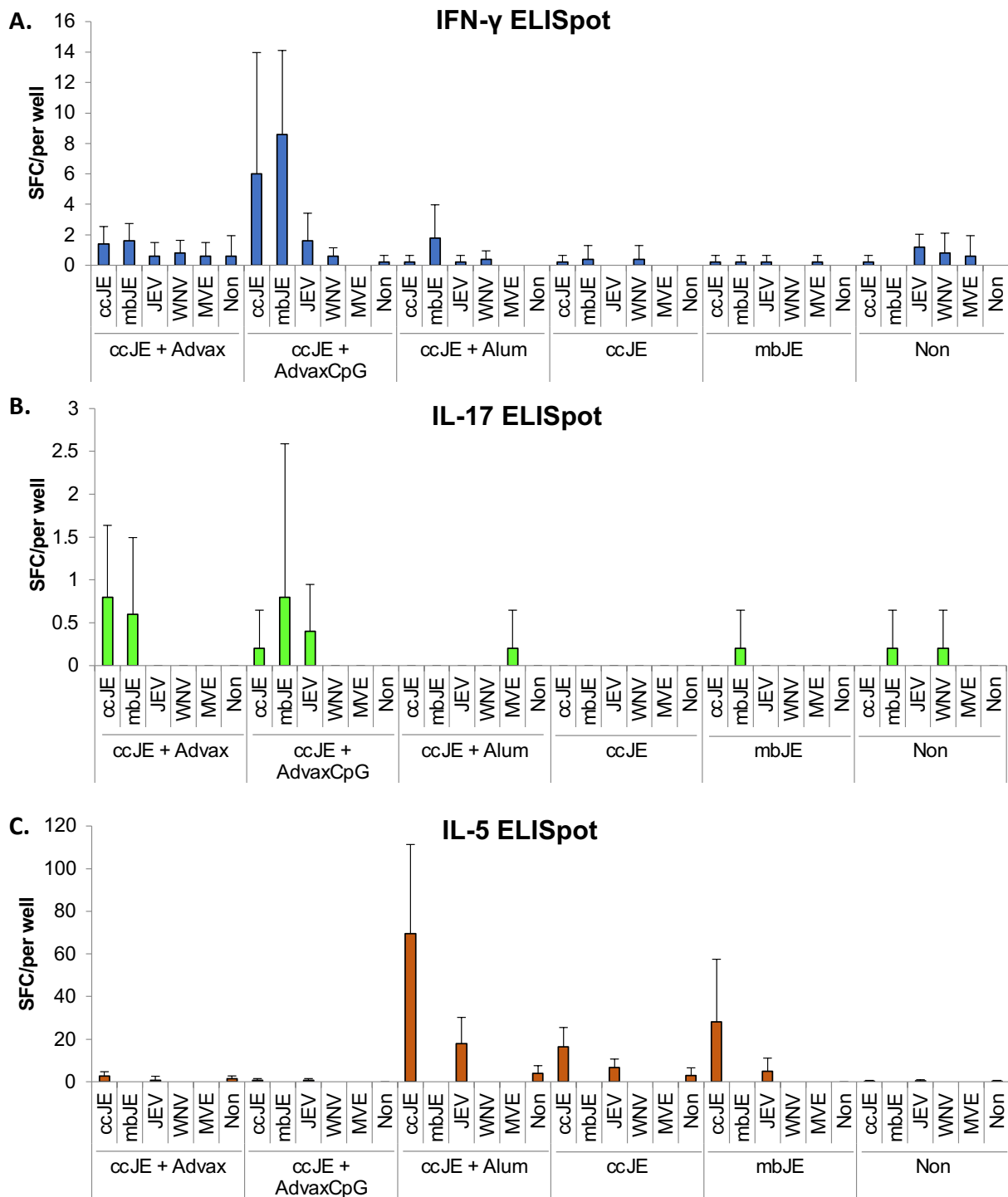
**An Advax-adjuvanted inactivated cell-culture Japanese
encephalitis vaccine induces broadly neutralising anti-
flavivirus antibodies and T-cell immunity and provides single
dose protection**

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Supplementary Materials

Immunised Mouse Sera	Challenge Virus				
	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.



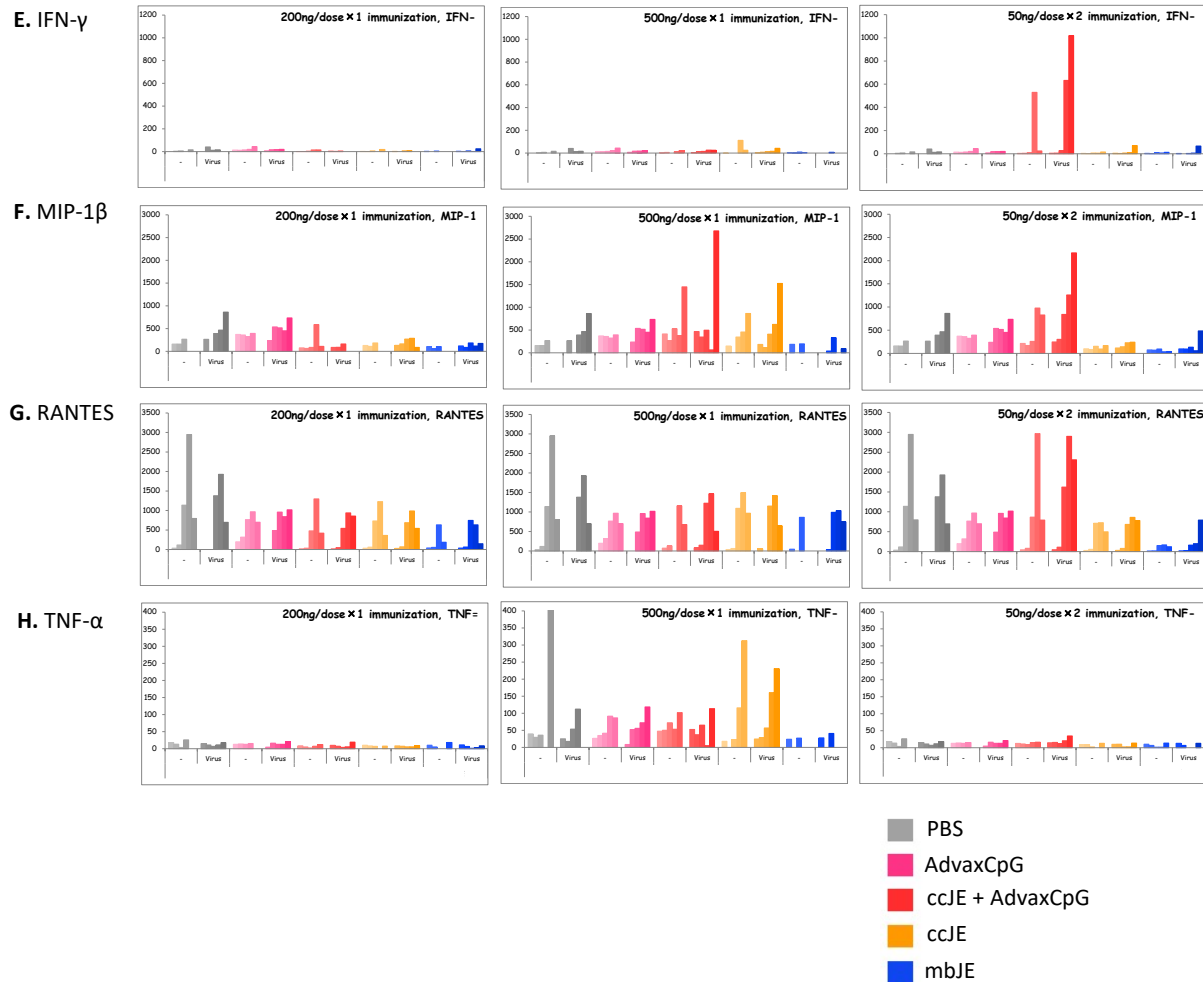
Supplementary Figure 1: Advax with CpG induces strong IFN- γ cellular response. Four-week-old C57BL/6 mice were immunised intramuscularly with mbJE or ccJE alone or with CpG or AdvaxCpG or Alum twice 3 weeks apart with a vaccine antigen dose of 50ng. Spleens were collected 3 weeks after the last immunisation. 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).

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Figure 1 consists of three bar charts showing the effect of Eotaxin on viral titers in the lungs of mice. The y-axis represents viral titer (log₁₀ copies/ml) and the x-axis represents the condition. The legend indicates that the bars represent viral titers in the lungs.

- 200ng/dose x1 immunization, Eotaxin:** The y-axis ranges from 0 to 400. The x-axis shows conditions: -, Virus, -, Virus, -, Virus, -, Virus, -, Virus. The bars show viral titers for each condition. The highest titer is observed in the 'Virus' condition (approx. 350 log₁₀ copies/ml).
- 500ng/dose x1 immunization, Eotaxin:** The y-axis ranges from 0 to 400. The x-axis shows conditions: -, Virus, -, Virus, -, Virus, -, Virus, -, Virus. The bars show viral titers for each condition. The highest titer is observed in the 'Virus' condition (approx. 350 log₁₀ copies/ml).
- 50ng/dose x2 immunization, Eotaxin:** The y-axis ranges from 0 to 400. The x-axis shows conditions: -, Virus, -, Virus, -, Virus, -, Virus, -, Virus. The bars show viral titers for each condition. The highest titer is observed in the 'Virus' condition (approx. 350 log₁₀ copies/ml).

Figure 1 consists of three bar charts showing the number of cells per sample for various cell types in three immunization regimens: 200ng/dose x1, 500ng/dose x1, and 50ng/dose x2. The y-axis represents the number of cells per sample (0 to 250). The x-axis shows cell types: -, Vlnst, -, Vlnst, -, Vlnst, -, Vlnst, -, Vlnst. The 50ng/dose x2 regimen shows a significant increase in Vlnst cells compared to the other two regimens.



Supplementary Figure 2: Cytokine production T-cells in single and two dose ccJE immunised mice AdvaxCpG. 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 splenocytes were isolated restimulated with or without virus. Cytokine production was measured using Bio-Plex Mouse Cytokine 23-plex Assay.