

Supplementary Table 1: Summary of Reactogenicity Events by Group and Time of Onset in Entebbe

Group	A (n=39)		B (n=11)	
	Onset Post-Vaccination			
Event	30 Minutes		3-14 days	
Pain	5 (13%)	1 (10%)	6 (15%)	0
Headache	3 (8%)	0	8 (20%)	1 (10%)
Malaise	3 (8%)	0	4 (10%)	0
Nausea	2 (5%)	0	3 (8%)	0
Induration	2 (5%)	0	2 (5%)	0
Fatigue	1 (3%)	0	0	1 (10%)
Fever	0	1 (10%)	3 (8%)	0
Myalgia	0	0	2 (5%)	0
Tenderness	0	0	2 (5%)	0
Erythema	0	0	1 (3%)	1(10%)
Total Events	16	2	31	3

Reactogenicity Event Summary in Group A (First Vaccination) and Group B (Boost Vaccination)

Overall, 27 of the 50 (54 %) vaccinated volunteers (23 from group A) reported 52 local and systemic reactogenicity events. Within 30 minutes post vaccination, 16 volunteers (32%) reported 18 events. Between 3 and 14 days post vaccination, 20 volunteers (40%) reported 34 events. All events were mild and all resolved within 8 days of onset. None of the reactogenicity events were unexpected based on the known safety profile of the YF-17D vaccine. None of the events interfered with daily activities. YF-17D naïve volunteers (Group A) who were vaccinated during the study reported more reactogenicity events compared with those who were boosted (Group B).

Supplementary Table 2: Summary of Adverse Events by Group in Entebbe

Group	A	B
AE	n=39	n=11
Neutropenia (Grade 1-3)	15 (38%)	3(30%)
URTI	5(13%)	0
Conjunctivitis	0	0
Tonsillitis	0	1(10%)
Anaemia	2(5%)	0
Increased ALT	1(3%)	0
Chicken Pox	1(3%)	0
Malaria	1(3%)	0
Headache	1(3%)	0
Thrombocytopenia	1(3%)	0
Gastritis	1(3%)	0
Total Events	28	4

Adverse Event (AE) Summary in Group A (First Vaccination) and Group B (Boost Vaccination)

Overall, 31 of 50 volunteers reported at least one AE during the course of the study. Out of the 35 AEs reported, 13 were clinical events while 22 were laboratory events. All AEs due to laboratory abnormalities were not clinically significant and apart from anaemia were self-limiting. All AEs reported in this study resolved without sequelae.

Supplementary Table 3: Adverse events surveillance in study volunteers from Lausanne

A total of 50 YF-17D immunized volunteers were reassessed at 4 visits: day 3, day 7, week 8 and ~ 1 year. Overall 43 (86%) were first-time vaccinees by contrast to 7 (14%) reimmunized subjects. In addition to YF-17D immunization, 19 (38%) individuals received one or more concomitant viral vaccines according to their trip destination needs and vaccination history. Frequency of other immunizations at day 0 were as follow:

Poliomyelitis / Poliorix® or Revaxis® = 12

Hepatitis A / Epaxal® = 14

Hepatitis B / Engerix® = 4

Combined Hepatitis A+B / Twinrix® = 5

Mumps-Measles-Rubella / Priorix® = 7

Varicella / Varilix® = 1

African meningitis/ Mencevax ACWY= 5

Serious and none-mild adverse events were recorded in 3 volunteers (6%). Briefly, 2 presented mild-moderate adverse events and 1 volunteer was hospitalized for pneumonia treatment. All three subjects were first-time recipients of yellow fever vaccine.

Adverse events reported in YF-vaccine recipients from Lausanne: 12 months follow-up.

Volunteer	Age	Gender	Adverse event	Time of onset	Concomitant vaccines
05	29	M	Flu-like symptoms T :39°C	Day 4	none
10	45	M	Injection site erythema and arm pain	Day 1	none
28	28	F	Recurrent guttate psoriasis	Week 16	Mencevax ®ACWY Malarone prophylaxis
38	43	F	Community-acquired pneumonia	Day 4	Poliorix Epaxal

Medical information concerning volunteers experiencing reported adverse events

YF-05

No medical history of allergy or pre-existing condition. Following day 4 onset of headache, sore throat and abdominal pain he reported persistent fever (max.39°C) from day 7 onto day 11 regardless intake of antipyretics. After resolution of fever, convalescence due to fatigue was present until week 2.

YF-10

Medical history of aspirin allergy and peptic ulcer disease. Injection site erythema and arm pain resolved by day 3 spontaneously.

YF-38

History of chronic hepatitis B, on medical treatment for hypertension and diabetes type 2. According to the hospital records, community-acquired pneumonia was confirmed at day 4 and resolved at day 9 after 5 days of hospitalization.

Supplementary Table 4: Comparison of YF-17D neutralizing antibody titers within subgroups in Entebbe

Group Comparisons	P value	P value
Male/Female	0.71	NS*
Neutropenia Yes/No	0.69	NS
Viral Load Yes/No	0.78	NS

*NS= not significant

Naïve vaccinees in Entebbe did not show significant differences in neutralizing antibody titers when comparing gender, neutropenia status, or detectable viremia. Therefore, these parameters were not impacting the response to the YF-17D vaccine.

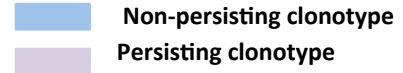
Supplementary Table 5: Correlation between hematological data at baseline in Entebbe and YF-17D neutralizing antibody titers

	Basophils	Eosinophils	Hematocrit	Hemoglobin	Lymphocyte
Spearman r	-0.1470	-0.0575	0.0514	0.0725	-0.1283
P value	0.3718	0.7281	0.7560	0.6611	0.4364
P value	NS*	NS	NS	NS	NS

	Monocytes	Neutrophils	Platelets	RBC	WBC
Spearman r	0.5753	0.1772	0.1821	-0.1321	0.0773
P value	0.0001	0.2804	0.2673	0.4227	0.6401
P value	Significant	NS	NS	NS	NS

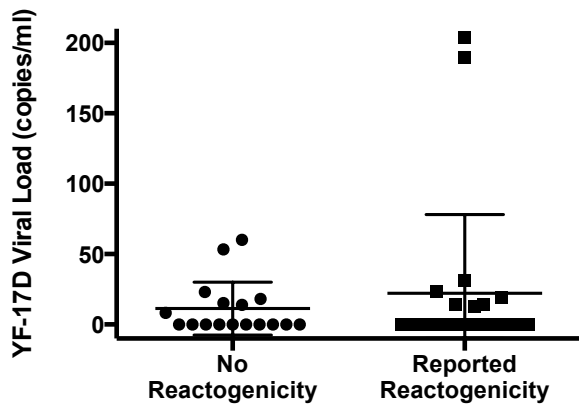
*NS= not significant

Supplementary Table 6: TCR Vbeta usage of the A2/NS4B-specific CD8 T cells at two time points in Lausanne and Entebbe.

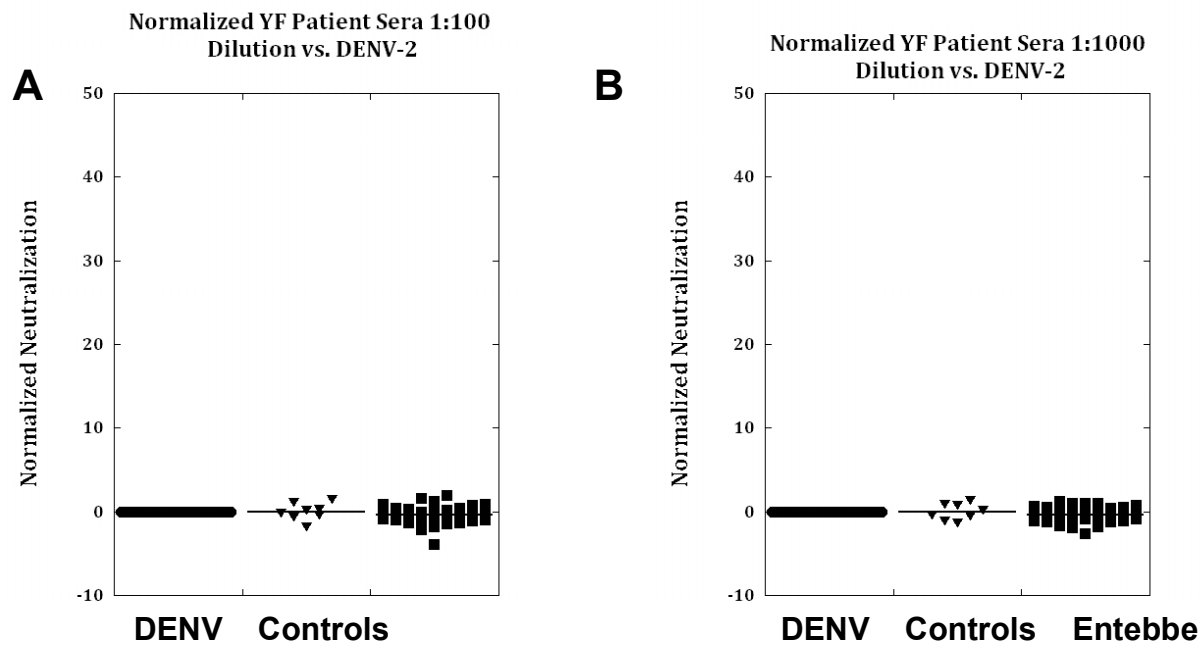


	SUBJECT	VISIT	TRBV4-1	TRBV5-5	TRBV28	TRBV3	TRBV14	TRBV19	TRBV5-1	TRBV18	TRBV30	TRBV6-5	TRBV6-6	TRBV12	TRBV5-6	TRBV10	TRBV20	TRBV9	TRBV11-2	TRBV13	TRBV2	TRBV25	TRBV27	TRBV4-3	TRBV6-2-3	TRBV29
Lausanne	1	W8	-	-	3.72	-	-	-	13.50	-	-	-	-	-	-	-	-	25.90	-	-	-	-	7.75	-	-	-
	1	1Y	-	-	3.92	-	-	-	7.14	-	-	-	-	-	-	-	18.40	34.30	-	-	-	-	-	-	-	-
	14	W8	-	-	-	-	-	-	9.93	-	-	-	-	-	-	15.50	4.31	20.90	-	-	-	-	-	-	-	-
	14	1Y	-	-	-	-	-	-	6.91	-	-	-	6.93	-	-	-	50.40	-	-	-	-	-	-	-	-	-
	27	W8	-	-	-	-	-	-	-	6.54	-	-	-	-	-	-	-	9.40	-	-	-	-	-	-	-	6.22
	27	1Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28.80	-	4.24	-	-	-	-	-	5.20
	30	W8	-	-	-	-	-	-	-	-	-	-	4.74	-	-	-	5.21	11.80	-	-	-	-	-	5.55	-	8.11
	30	1Y	-	-	4.01	-	-	-	-	-	-	-	-	-	-	8.57	3.35	14.60	-	-	-	-	-	5.70	-	6.05
	43	W8	-	-	-	-	-	-	-	5.24	-	-	-	-	-	-	-	62.70	-	-	-	-	-	3.80	-	4.12
	43	1Y	-	-	-	-	-	-	-	-	-	-	-	12.00	-	-	-	15.40	-	-	-	12.40	-	-	-	29.30
	44	W8	-	-	-	-	-	-	-	6.19	-	-	-	-	-	-	10.90	3.53	3.23	-	-	-	-	-	-	-
	44	1Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.32	-	9.21	-	15.90	-	-	6.51	8.59	-
	45	W8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.08	14.80	-	-	-	-	-	7.57	6.43	-
	45	1Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.43	14.60	-	-	-	-	-	3.33	-	9.46
	49	W8	-	-	5.97	-	-	-	-	-	-	-	-	-	-	-	-	4.82	13.60	-	-	-	-	-	-	20.10
49	1Y	-	-	7.09	-	-	-	-	3.43	-	-	7.15	-	-	-	-	8.09	7.55	-	-	-	-	-	6.31	43.10	
Entebbe	7	V7	-	-	9.22	-	-	-	-	-	-	-	-	-	-	-	10.50	-	-	-	-	-	5.87	-	3.58	
	7	V8	-	-	-	-	6.86	-	4.63	-	-	-	-	-	-	-	3.15	37.90	-	-	-	-	-	-	3.14	
	37	V7	-	-	-	-	-	-	3.13	-	-	-	-	-	-	13.00	-	9.96	-	-	-	-	-	-	8.79	
	37	V8	-	-	-	-	4.86	-	-	-	24.60	14.00	-	-	-	-	-	13.20	-	-	-	-	-	-	-	
	42	V7	-	-	-	-	-	-	8.27	-	-	-	-	-	-	-	-	14.70	-	10.90	-	-	-	-	18.20	
	42	V8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.83	80.80	-	-	-	8.44	
	48	V7	-	-	-	-	6.21	-	-	-	4.31	-	-	-	-	4.11	3.60	11.50	-	4.52	17.40	-	-	-	6.38	
	55	V7	-	-	-	-	-	4.23	-	-	8.25	-	-	-	-	-	4.77	15.20	-	-	5.18	-	-	-	24.20	
	55	V8	-	-	7.15	-	-	-	-	-	-	-	-	-	-	-	4.84	12.10	-	-	-	-	-	-	13.80	3.60
	Entebbe Boosted	69	V2	-	-	-	-	-	-	-	-	-	-	-	3.01	-	-	16.10	10.20	-	-	3.61	-	-	-	87.40
69		V7	-	-	-	-	-	-	4.27	-	-	-	-	-	-	-	11.20	3.00	-	4.07	-	-	-	-	12.40	
69		V8	-	-	-	-	-	-	4.68	-	12.60	-	-	-	-	-	-	-	-	-	-	8.53	-	-	10.90	
75		V2	-	-	-	-	-	-	4.68	-	-	-	-	6.55	-	-	-	-	-	-	-	-	4.87	-	7.48	
75		V7	-	3.18	-	-	-	-	3.81	-	-	-	-	-	-	-	8.43	10.70	-	7.15	-	-	-	-	-	
75	V8	-	7.71	-	-	-	3.30	4.88	-	-	-	-	-	-	-	-	12.10	9.60	-	-	-	-	-	12.60	4.51	

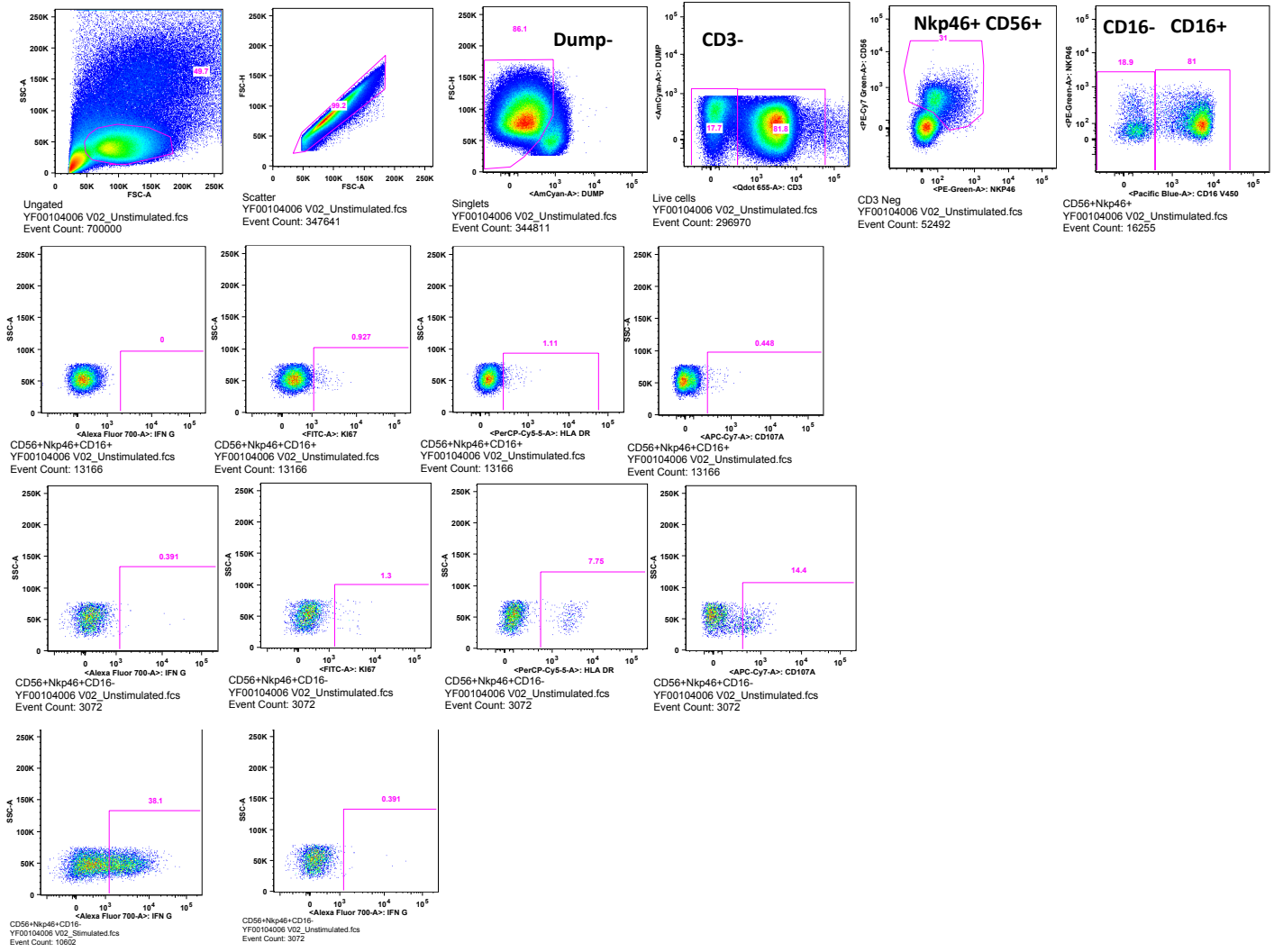
Supplementary Figure 1: No association between YF-17D viral load and reactogenicity reported either 30 minutes or in the follow up 3-14 days after vaccination in the Entebbe cohort .



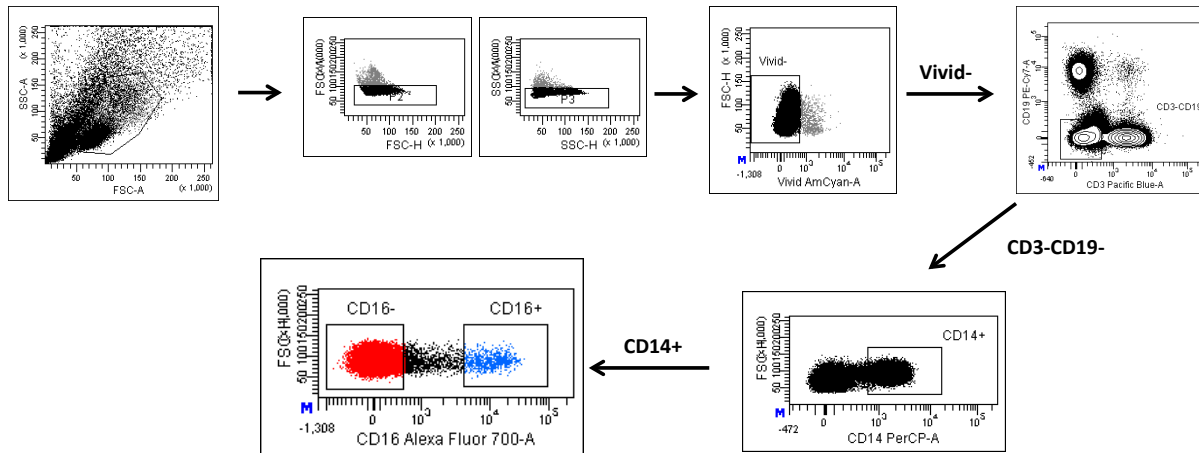
Supplementary Figure 2: Normalized neutralizing activity against DENV-2 of the sera of vaccinees from Entebbe at baseline and sera from controls at dilutions of 1:100 (A) and 1:1000 (B).



Supplementary Figure 3: Gating strategy for the NK cell populations analysis and monocytes by flow cytometry.

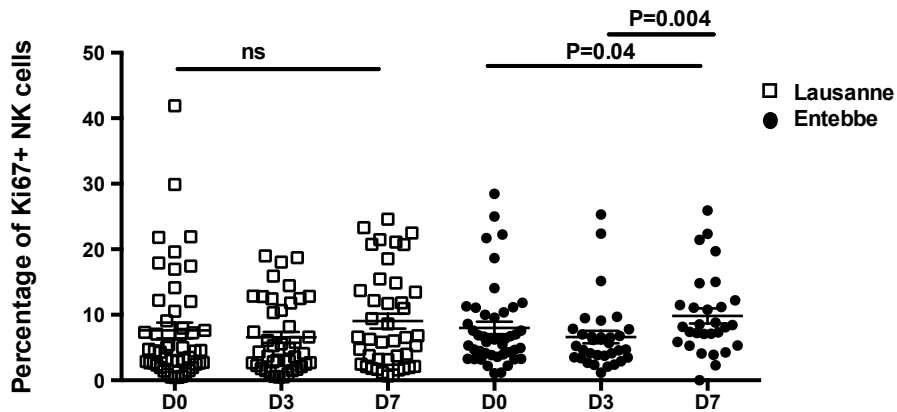


Monocyte subsets

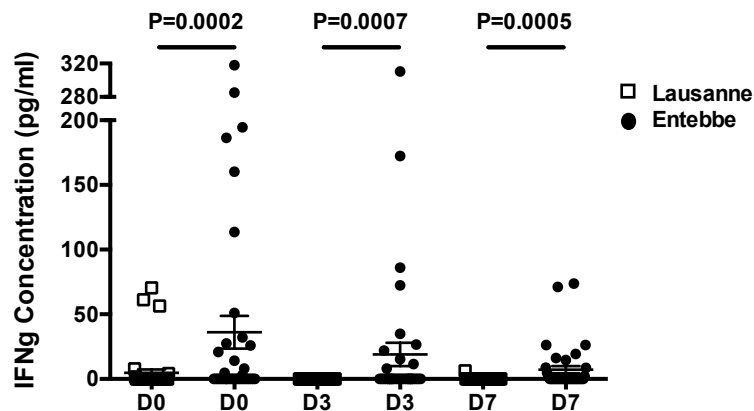


Supplementary Figure 4: (A) Frequency of Ki67+ NK cells at day 0, 3 and 7 after vaccination in Lausanne and Entebbe. (B) IFN γ concentration in the supernatant of PBMCs from Lausanne and Entebbe at day 0, 3 and 7 after vaccination incubated without any stimulation overnight. (C) Expression of PD-L1 and HLA-DR on monocytes CD14+ CD16- and CD14+ CD16+.

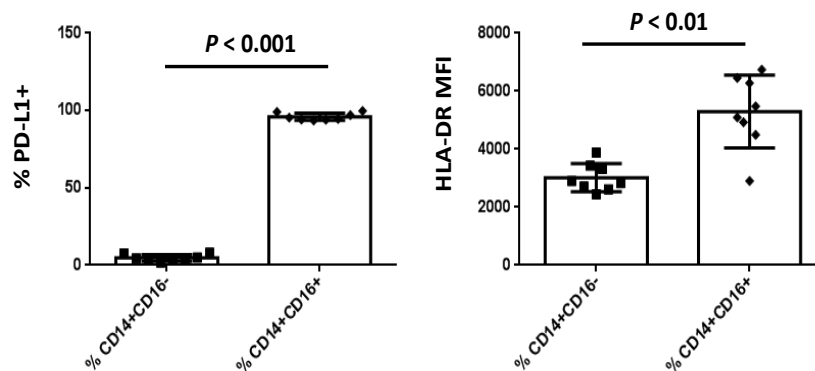
A



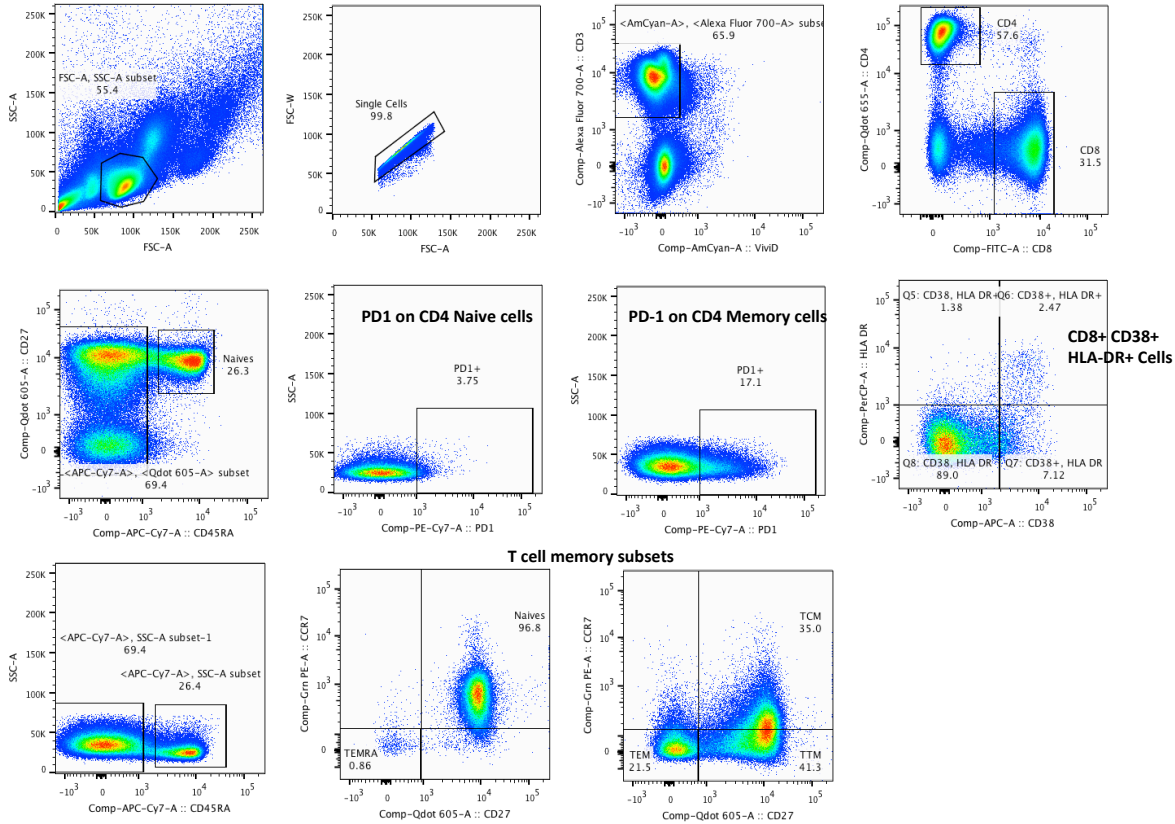
B



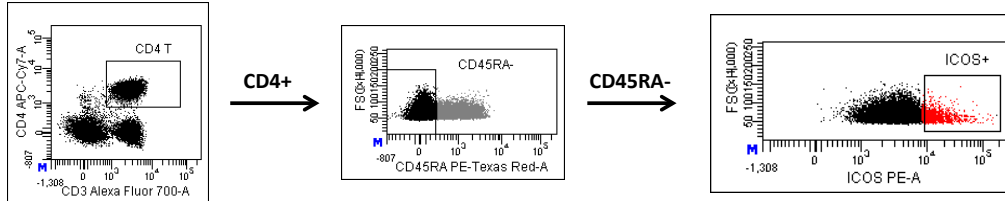
C



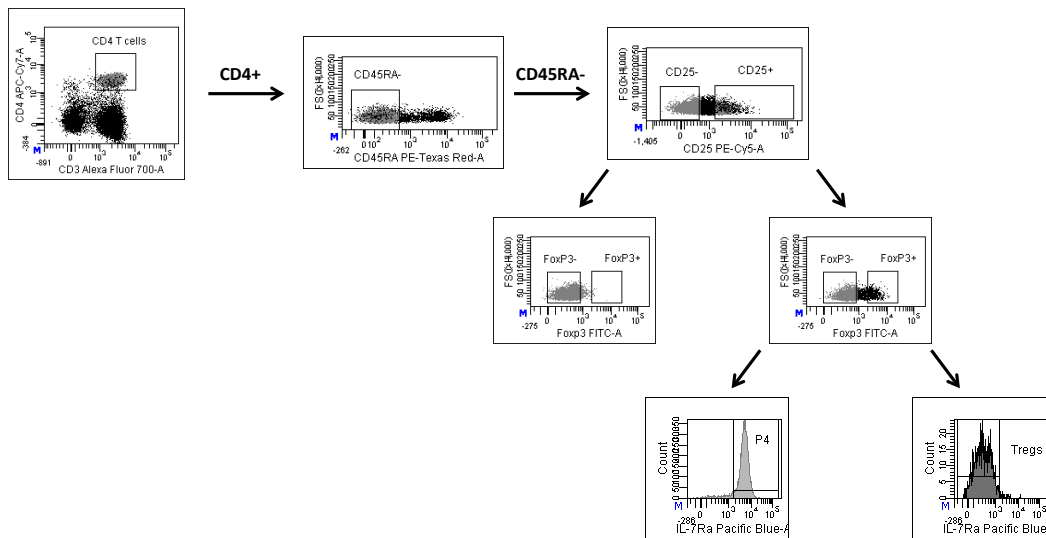
Supplementary Figure 5: Gating strategy for the T cell population analysis by flow cytometry.



Memory CD4+ICOS+ T cells

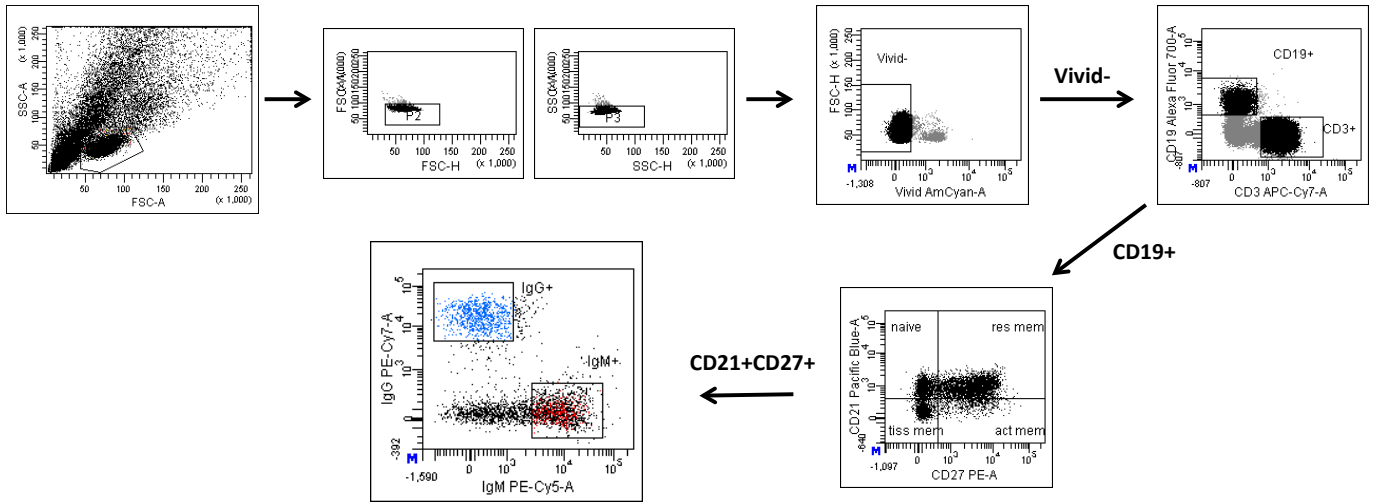


CD4+ Treg cells

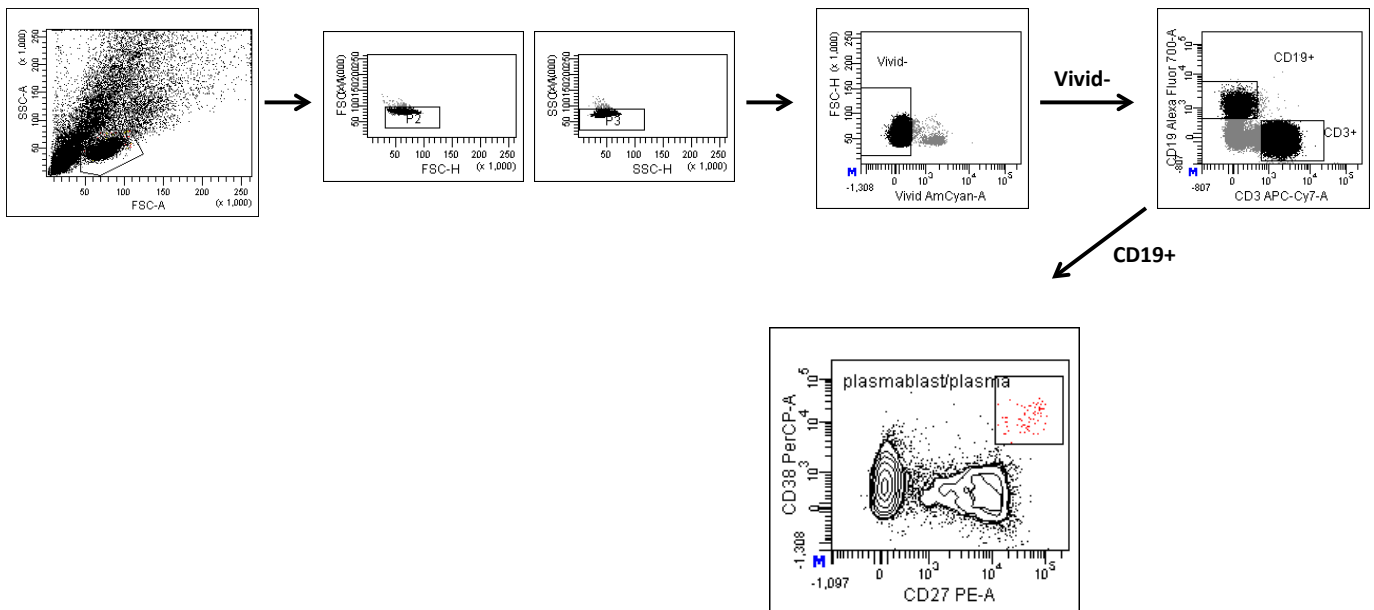


Supplementary Figure 6: Gating strategy for the B cell population analysis by flow cytometry.

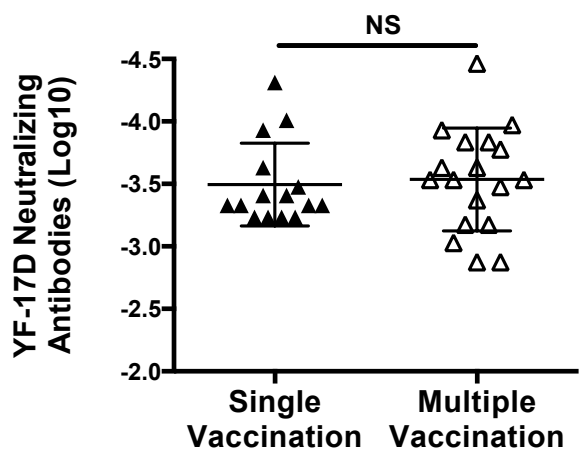
B cell subsets



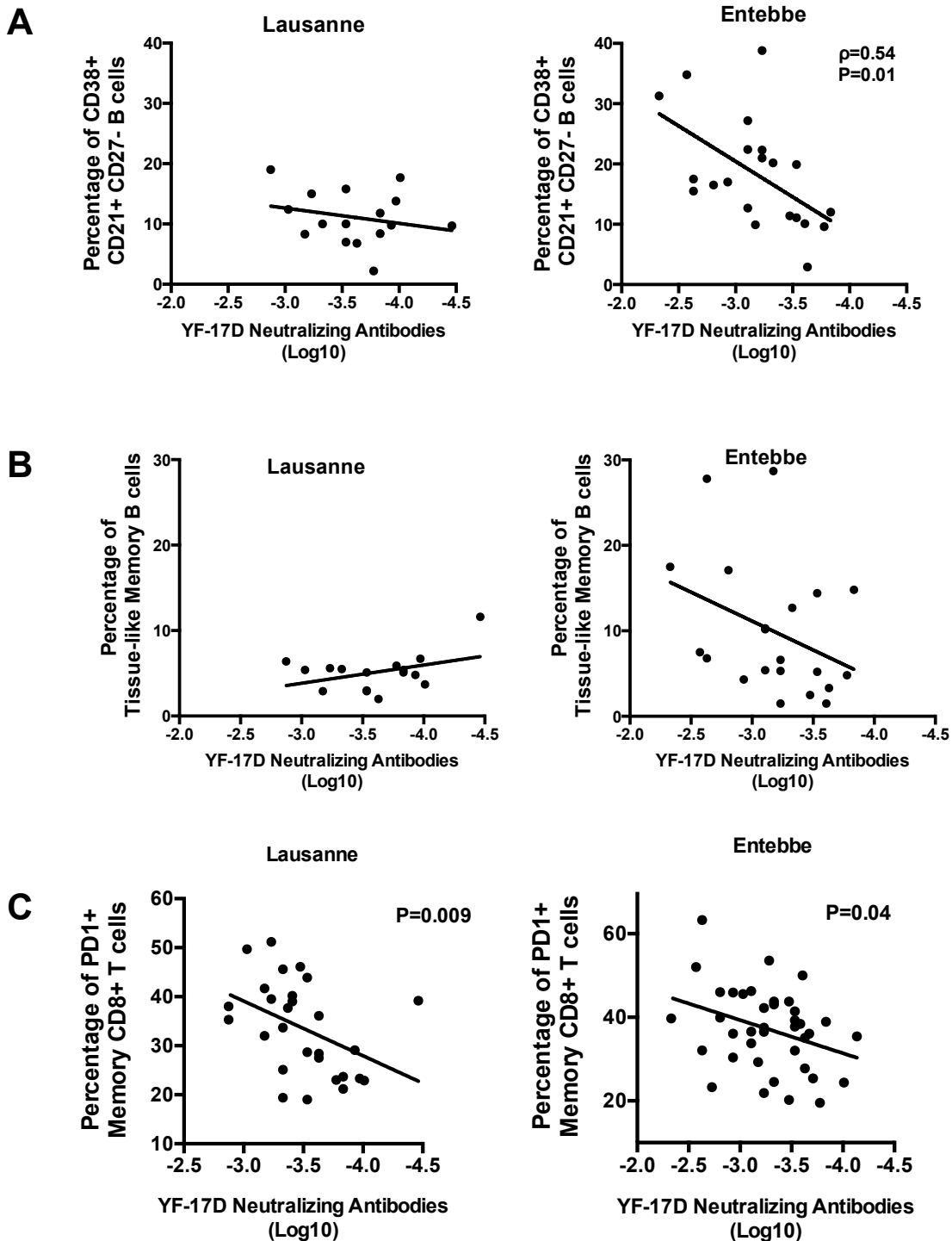
Plasma Cells/Plasmablasts



Supplementary Figure 7: No difference of neutralizing antibody titers in individuals receiving YF-17D alone or concomitant with other vaccines in the Lausanne cohort.

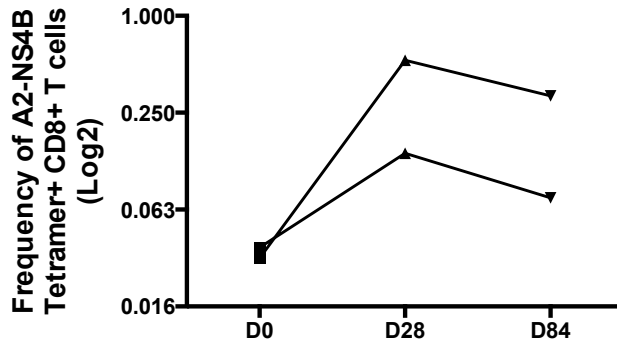


Supplementary Figure 8: Negative associations between baseline immune activation and YF-17D vaccine response. (A) Negative correlation between the frequency of CD38+ CD21+ CD27- B cells at baseline and the YF-17D NAb titers in the Lausanne and Entebbe cohorts. (B) Negative correlation between the frequency of tissue-like memory B cells at baseline and the YF-17D NAb titers in the Lausanne and Entebbe cohorts. (C) Negative correlation between the expression level of PD-1 on memory CD8 T cells at baseline and the YF-17D NAb titers in the Lausanne and Entebbe cohorts.

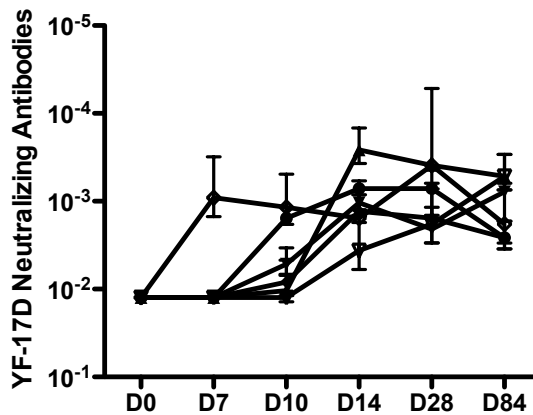


Supplementary Figure 9: Adaptive response to YF-17D vaccination in boosted vaccinees from Entebbe. Boosting of the A2/NS4B-specific CD8 T cell responses was observed in the two HLA-A2*0201 subjects receiving a second YF-17D vaccination (A). Neutralizing antibody boost against YF-17D was detected both in subjects with undetectable (B) and detectable (C) neutralizing antibody titers at baseline.

A



B



C

