

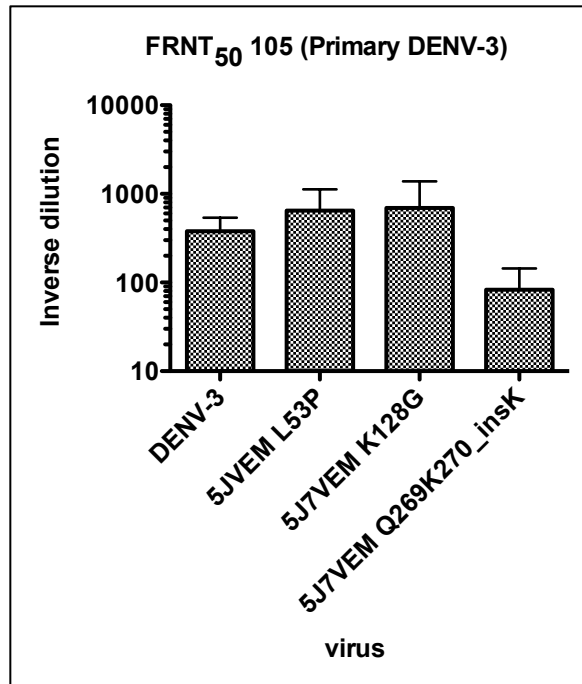
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exposure and year year collected	Primary DENV-3 50% Neutralization Titers			
	Donor 003	Donor 033	Donor 105	Donor 118
	Thailand 2001	India 2005	Thailand 2002	Nicaragua 2009
	2005	2006	2010	2010
DENV 1	1:30	<1:20	<1:20	1:60
DENV 2	1:87	<1:20	<1:20	1:32
DENV 3	1:338	1:426	0.1875	1:980
DENV 4	<1:20	1:25	<1:20	1:76

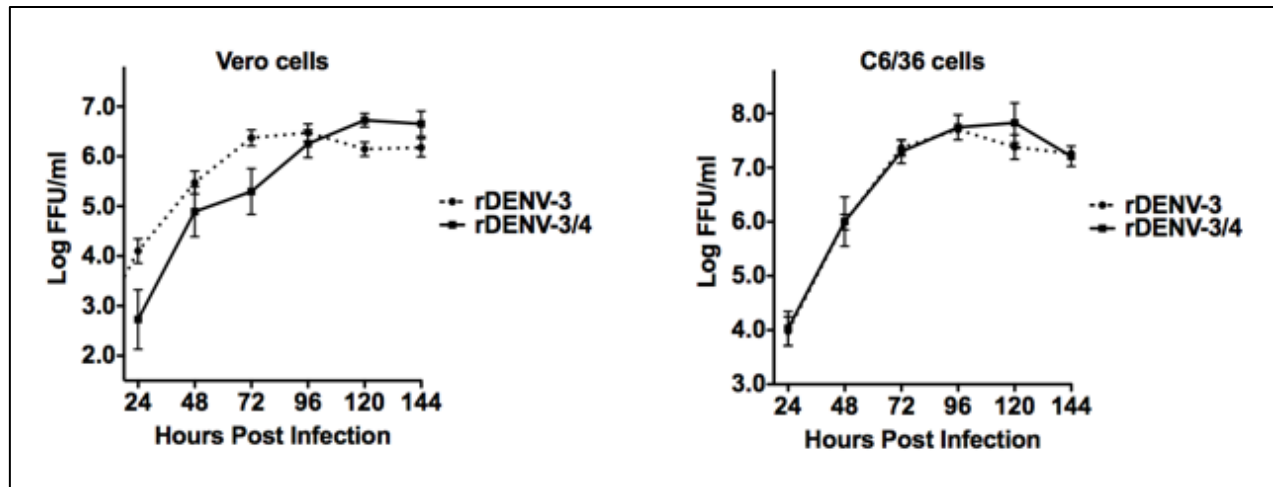
SI Table S1: Panel of previously characterized human primary DENV-3 immune sera. FRNT₅₀ titers were determined against prototype DENV viruses West Pac '74 (DENV-1), S-16803 (DENV-2), CH53489 (DENV-3) and TVP-360 (DENV-4).

Exposure and Year	Primary DENV-4 50% Neutralization Titers			
	Donor 102	Donor 97	Donor 98	Donor 99
	Honduras 2007	unknown	unknown	unknown
DENV 1	<1:20	<1:20	<1:20	<1:20
DENV 2	<1:20	<1:20	<1:20	<1:20
DENV 3	<1:20	<1:20	<1:20	<1:20
DENV 4	1:422	1:146	>1:1280	1:867

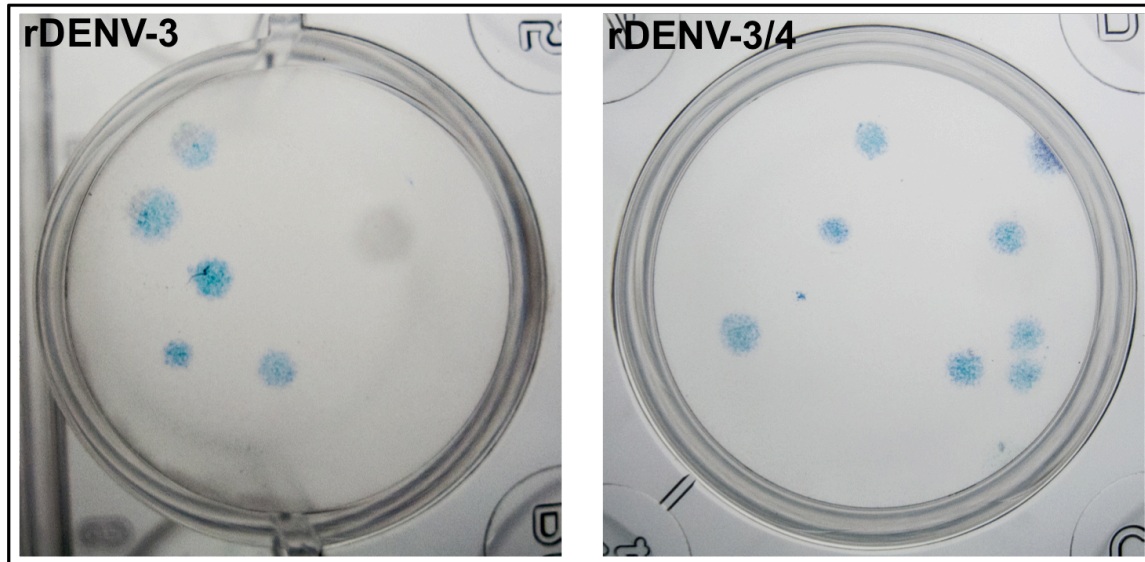
SI Table S2: Panel of previously characterized human primary DENV-4 immune sera. FRNT₅₀ titers were determined against prototype DENV viruses West Pac '74 (DENV-1), S-16803 (DENV-2), CH53489 (DENV-3) and TVP-360 (DENV-4).



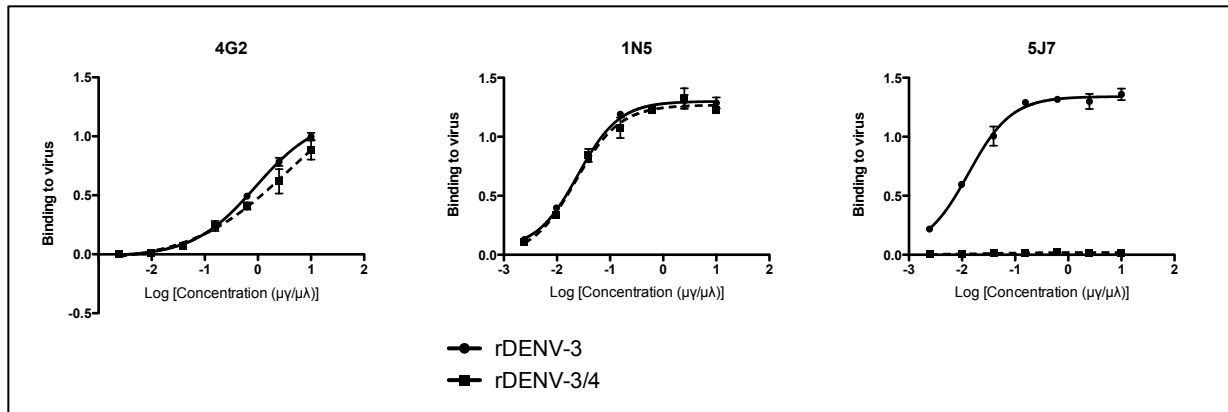
SI Figure S1: FRNT₅₀ values for the parental rDENV-3 infectious clone, parental rDENV-4 and rDENV-3/4 against donor 105 immune sera. The Y-axis shows serum dilution that neutralized 50% of input virus on a log scaled. X-axis shows input virus. Error bars show 95% CIs.



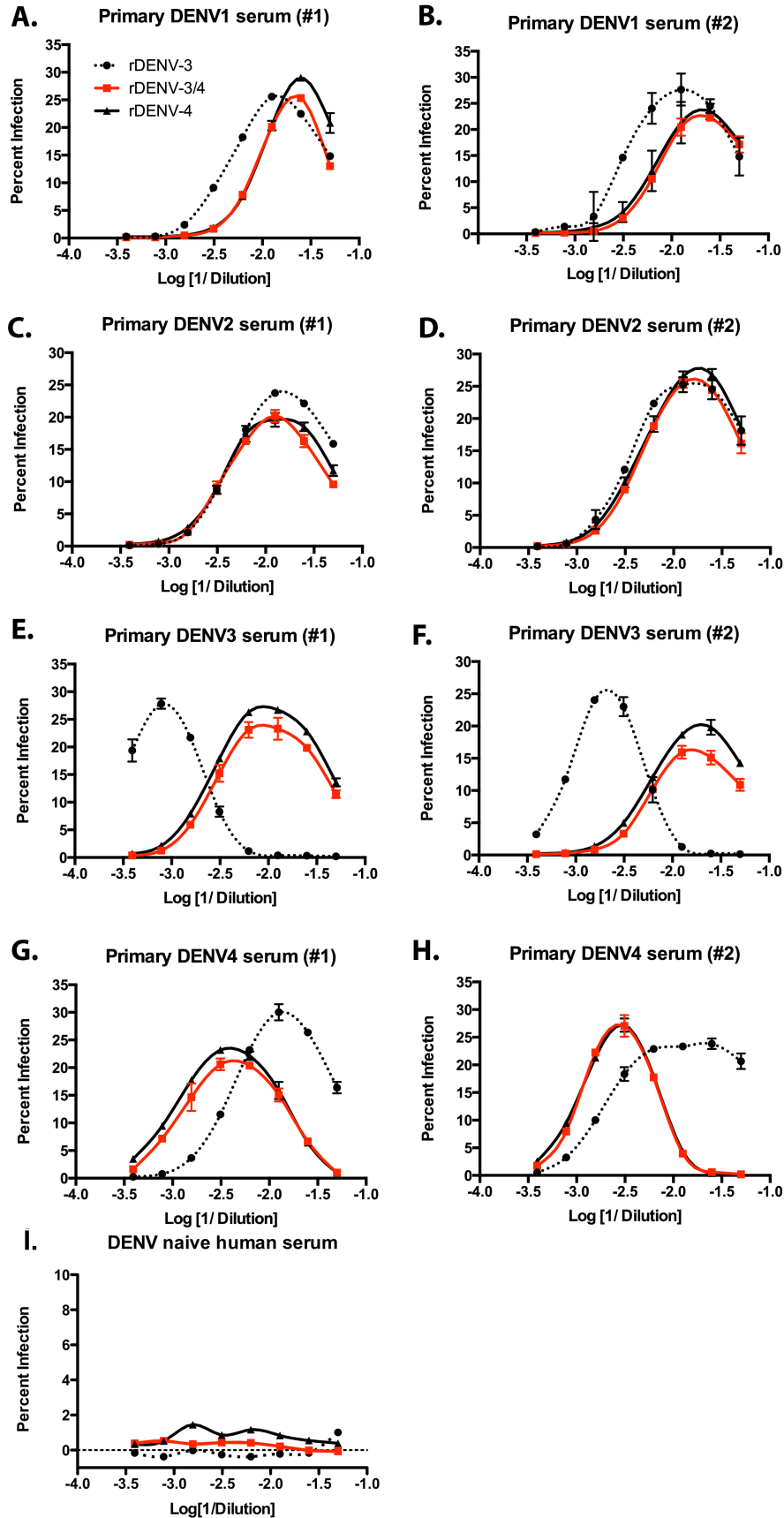
SI Figure S2: Vero E6 and C6/36 cells were inoculated at a multiplicity of infection (m.o.i) of 0.01 FFU. Cell culture supernatants were harvested at indicated times and the virus released from the infected cells was quantitated by immunofocus assay. Points show geometric mean titer (GMT) calculated from triplicate titrations. Error bars indicate 95% confidence intervals for each GMT. No focus forming units were observed at 0 hrs.



SI Figure S3: Focus formation by rDENV-3 and rDENV-3/4. Approximately 5 – 10 focus forming units/well were inoculated onto 90% confluent Vero E6 cells, overlaid with 1% methylcellulose and incubated for 5 days. Cells were fixed and foci visualized by immunostaining with monoclonal antibody 4G2 followed by horse-radish peroxidase (HRP) conjugated goat-anti-mouse secondary antibody and addition of True Blue (KPL) HRP substrate.



SI Figure S4: ELISA binding of whole rDENV-3 and rDENV-3/4 by E specific monoclonal antibodies 4G2, 1N5 and 5J7. The Y-axis shows relative optical density (OD) and the X-axis shows antibody concentration.



SI Figure S5. Antibody Dependent Enhancement of parental and recombinant DENVs by human primary sera in K562 cells. All above viruses display similar enhancement curves with heterologous DENV1 and DENV2 primary immune sera. At high concentrations primary DENV3 immune sera enhanced the rDENV4 and the rDENV3/4 chimera, but not rDENV3. At high concentrations primary DENV4 immune sera enhanced rDENV3, but not rDENV4 or the rDENV3/4 chimera. Strongly neutralizing sera that failed to enhance at high concentrations did enhance at low concentrations when antibodies were diluted to sub-neutralizing levels. The X-axis shows inverse log fold-dilution of sera. The Y-axis shows percent of K562 cells infected with DENV at a given serum concentration (X-axis) as measured by flow-cytometry. I shows enhancement of DENV-3, DENV-4 and rDENV-3/4 by DENV naïve human serum. Error bars show standard error.