1 **SUPPLEMENTAL:**

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- 2 Table 1: Attenuation of recombinant viruses, expressed as peak titer differences for each
- 3 recombinant virus relative to wild type [log10(recombinant/WT)], estimated by linear
- 4 regression. Differences are expressed in log10(IU/mL).

Virus	Vero		C6/36	
	Difference	p value	Difference	p value
	from Wild		from Wild	
	Туре		Type	
WNVΔCtrl-1	-0.287	0.032	0.252	0.701
WNVΔCtrl-2a	-0.644	< 0.001	< 0.001	1.000
WNVΔCtrl-2b	-0.187	0.149	-0.527	0.426
WNVΔCtrl-3	-0.767	< 0.001	0.385	0.559
WNVΔVax-1	-0.290	0.031	-3.186	< 0.001
WNV∆Vax-2a	-0.603	< 0.001	-3.382	< 0.001
WNVΔVax-2b	-0.392	0.005	-5.897	< 0.001
WNVΔVax-3	-0.920	< 0.001	-4.969	< 0.001

6 Table 2: P values for two-sample t test of peak titers, control compared to vaccine viruses.

Virus	Vero	C6/36
WNVΔ1	0.975	0.049
WNV∆2a	0.604	0.001
WNV∆2b	0.361	< 0.001
WNV Δ3	0.220	< 0.001

- 8 Table 3: Mean differences in Vero cell plaque sizes for each recombinant virus relative to
- 9 wild type [Recombinant WT], estimated by linear regression. Differences in plaque
- diameters are expressed in mm, with negative values indicating smaller plaques.

Virus	Difference from	p value
	Wild Type	
WNV∆Ctrl-1	-0.310	0.093
WNVΔCtrl-2a	-1.600	< 0.001
WNVΔCtrl-2b	-1.810	< 0.001
WNVΔCtrl-3	-1.844	< 0.001
WNVΔVax-1	-0.673	< 0.001
WNVΔVax-2a	-0.979	< 0.001
WNVΔVax-2b	-0.906	< 0.001
WNVΔVax-3	-1.884	< 0.001

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12 Table 4: *P* values for two-sample *t* test of plaque sizes, control compared to vaccine viruses.

Virus	Vero
WNV∆1	0.219
WNV∆2a	0.028
WNV∆2b	0.001
WNV Δ3	0.644