

Supplemental Data: Dual-Adjuvant Mucosal Vaccine Leveraging Mast Cell and TLR9 Agonists for Protection Against Poxvirus Infection

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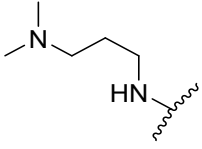
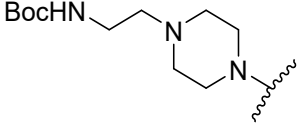
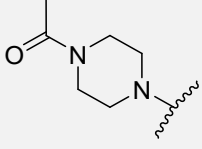
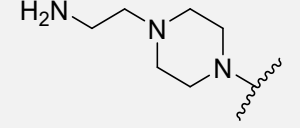
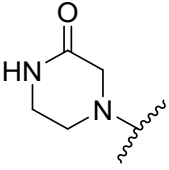
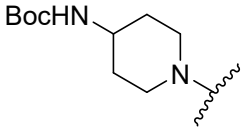
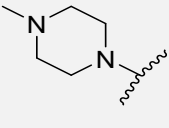
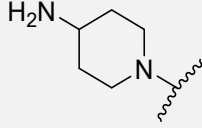
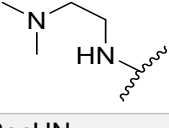
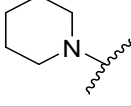
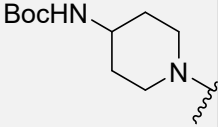
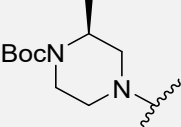
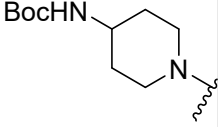
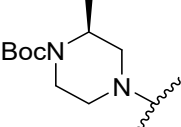
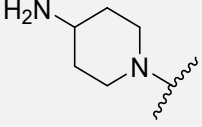
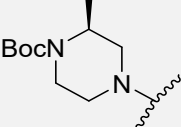
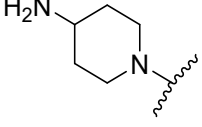
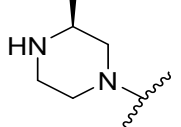
⁵Carborro High School, Chapel Hill, NC, US

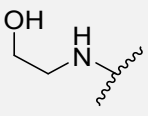
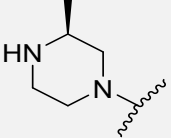
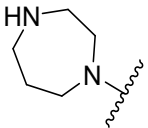
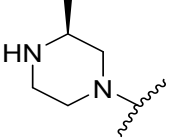
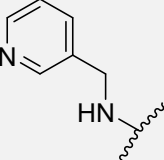
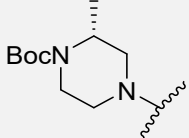
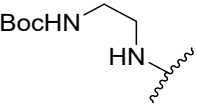
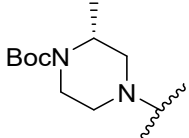
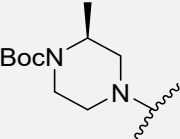
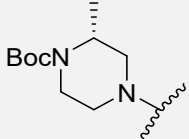
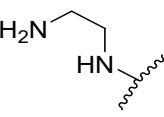
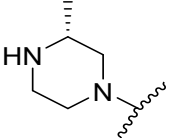
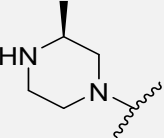
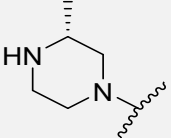
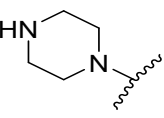
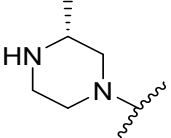
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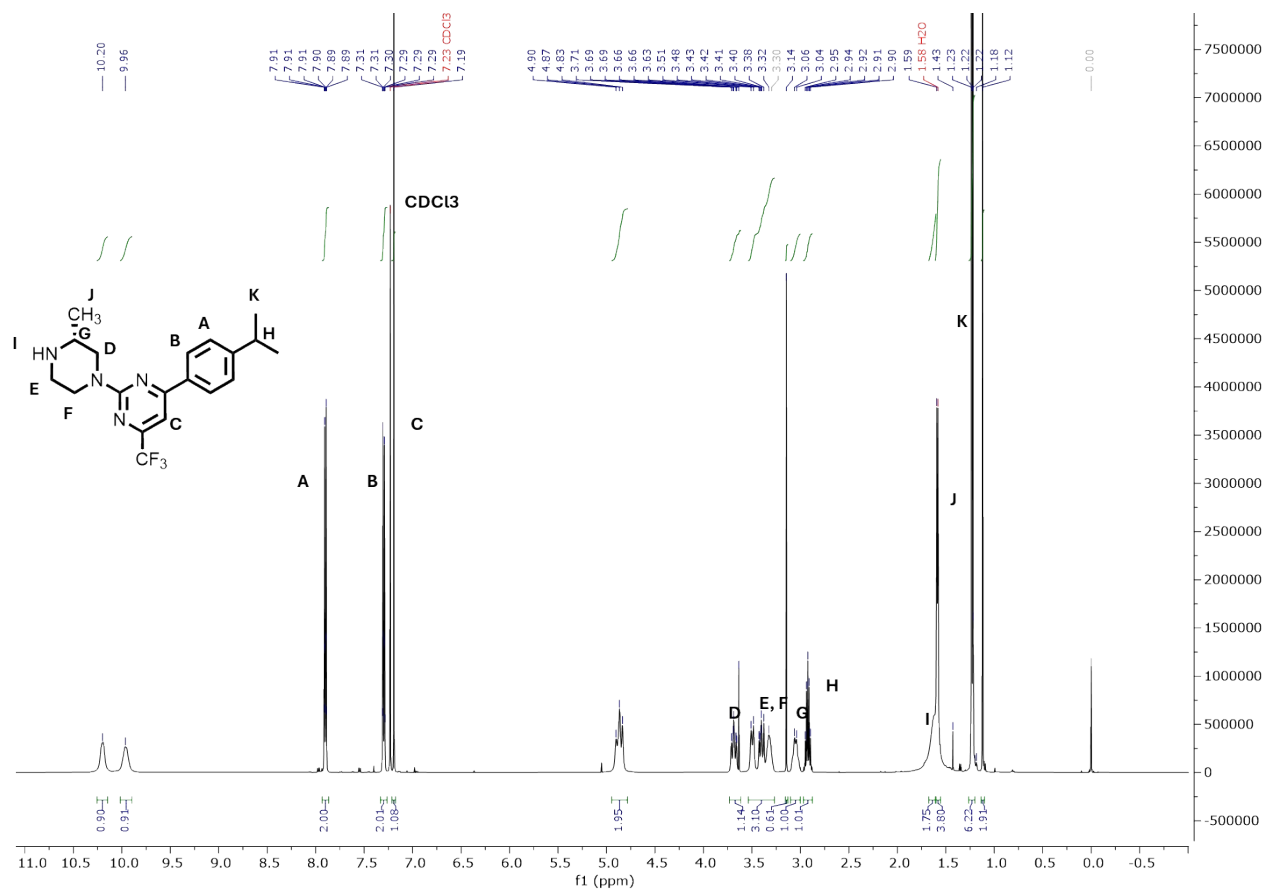
⁸Department of Microbiology and Immunology, School of Medicine, UNC, Chapel Hill, NC, USA



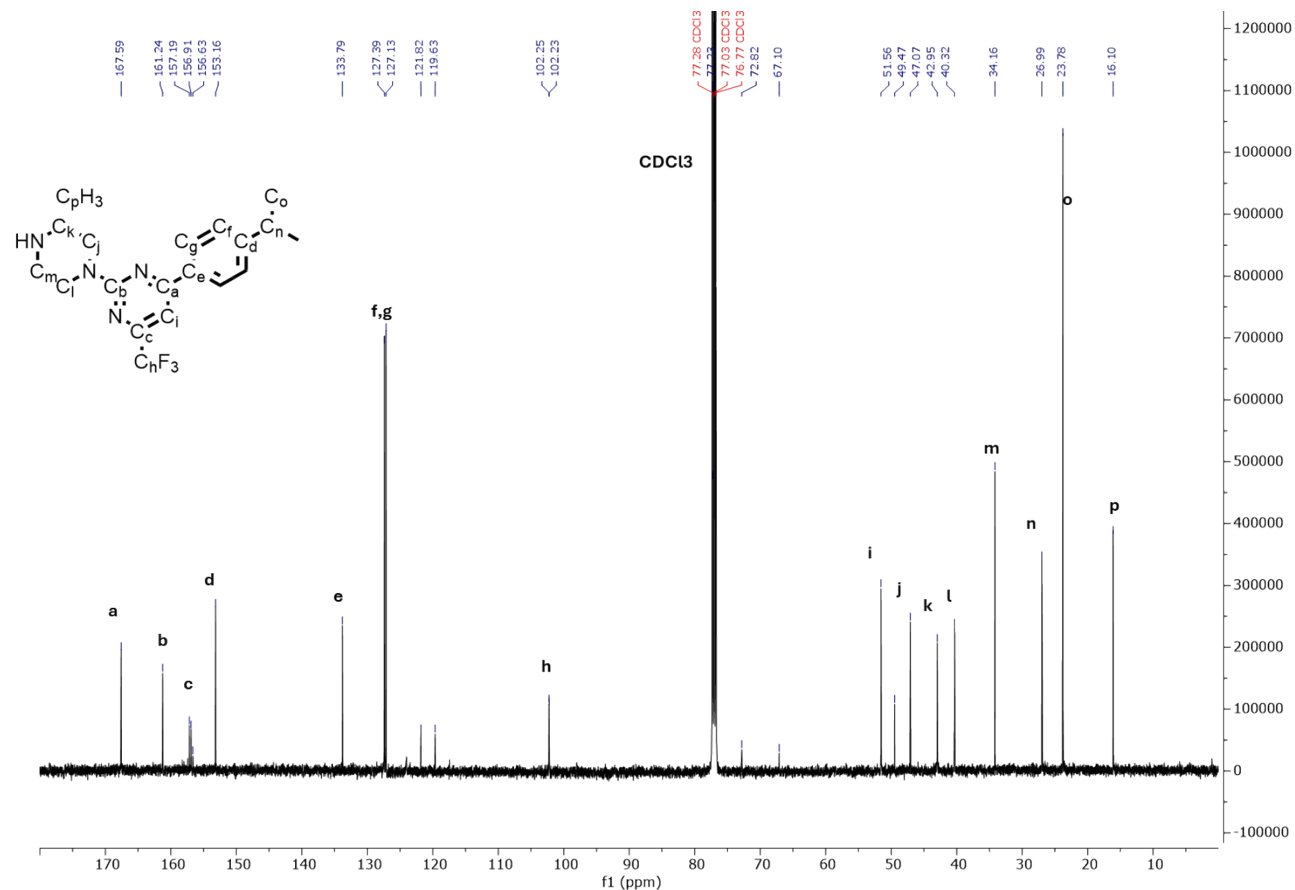
(Parent)				(Core scaffold)			
Entry	VAP reg #	R ₁	R ₂	Entry	VAP reg #	R ₁	R ₂
1	1136		Et	19	1166		Et
2	1137		Et	20	1167		Et
3	1138		Et	21	1169		MeO
4	1143		Et	22	1170		MeO
5	1144		Et	23	1171		Et
6	1145		EtO	24	1172		MeO
7	1146		<i>i</i> -Pr	25	1173		EtO
8	1151		EtO	26	1174		<i>i</i> -Pr
9	1153		<i>i</i> -Pr	27	1176		MeO

10	1154		Et	28	1177		EtO
11	1155		Et	29	1178		<i>i</i> -Pr
12	1156		Et	30	1180		MeO
13	1158		Et	31	1181		EtO
14	1159		Et	32	1182		<i>i</i> -Pr
15	1160		Et	33	1183		MeO
16	1161		Et	34	1184		EtO
17	1162		Et	35	1185		<i>i</i> -Pr
18	1164	4-Cl-Ph	4-Cl-Ph				

Supplemental Table 1. The 35 analogs of ST101036 following SAR analysis.

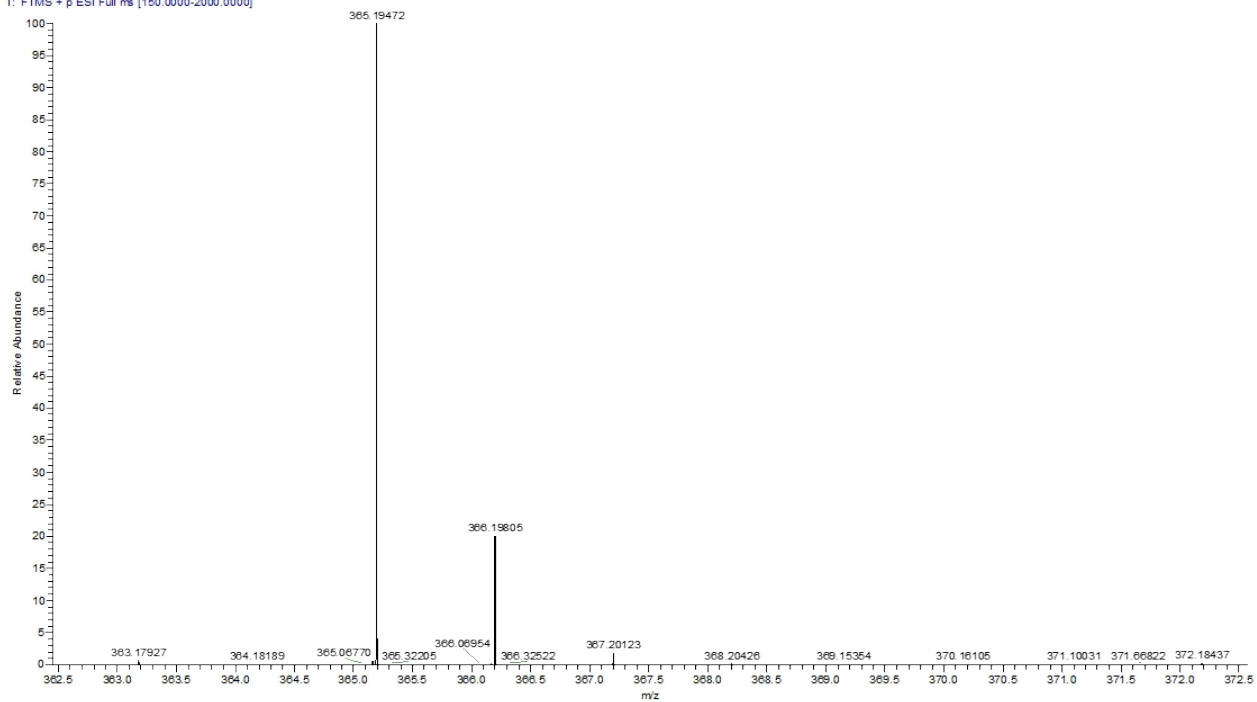


Supplemental Figure 1. ¹H-NMR spectrum of VAP-1185. ¹H NMR (500 MHz, CDCl₃): δ10.20 (s, 1H, N-H), 9.96 (s, 1H, N-H), 7.91 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.30 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.19 (s, 1H), 4.90–4.80 (m, 2H), 3.75–3.60 (m, 2H), 3.51–3.40 (m, 2H), 3.14 (s, 3H, N-CH₃), 3.08–2.90 (m, 2H), 1.59 (d, *J* = 6.8 Hz, 6H, CH(CH₃)₂), 1.43 (m, 1H, CH(CH₃)₂).

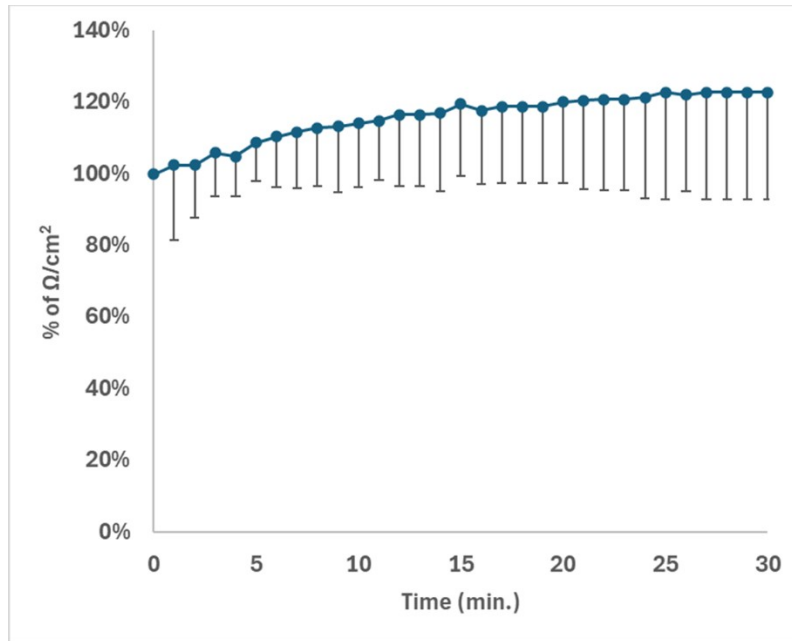


Supplemental Figure 2. ^{13}C -NMR spectrum of VAP-1185. ^{13}C NMR (100 MHz, CDCl_3): δ 167.59 (a), 161.24 (b), 157.19 (q, $J = 35.2$ Hz, c), 153.16 (d), 133.79 (e), 127.39 (f), 127.13 (g), 121.82 (q, $J = 274.5$ Hz, CF_3), 102.25 (h), 51.56 (i), 47.07 (j), 42.95 (k), 40.32 (l), 34.16 (m), 26.99 (n), 23.78 (o), 16.10 (p).

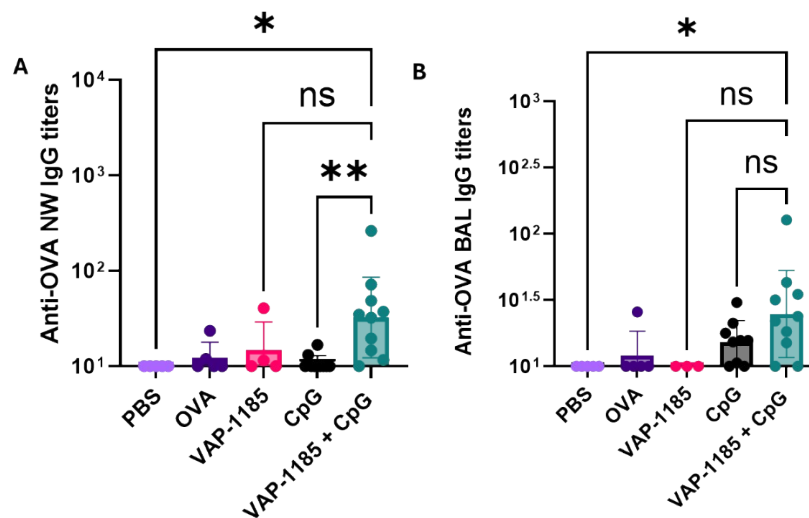
VAP-1185 #1-100 RT: 0.01-1.30 AV: 100 NL: 9.79E9
T: FTMS + p ESI Full ms [150.0000-2000.0000]



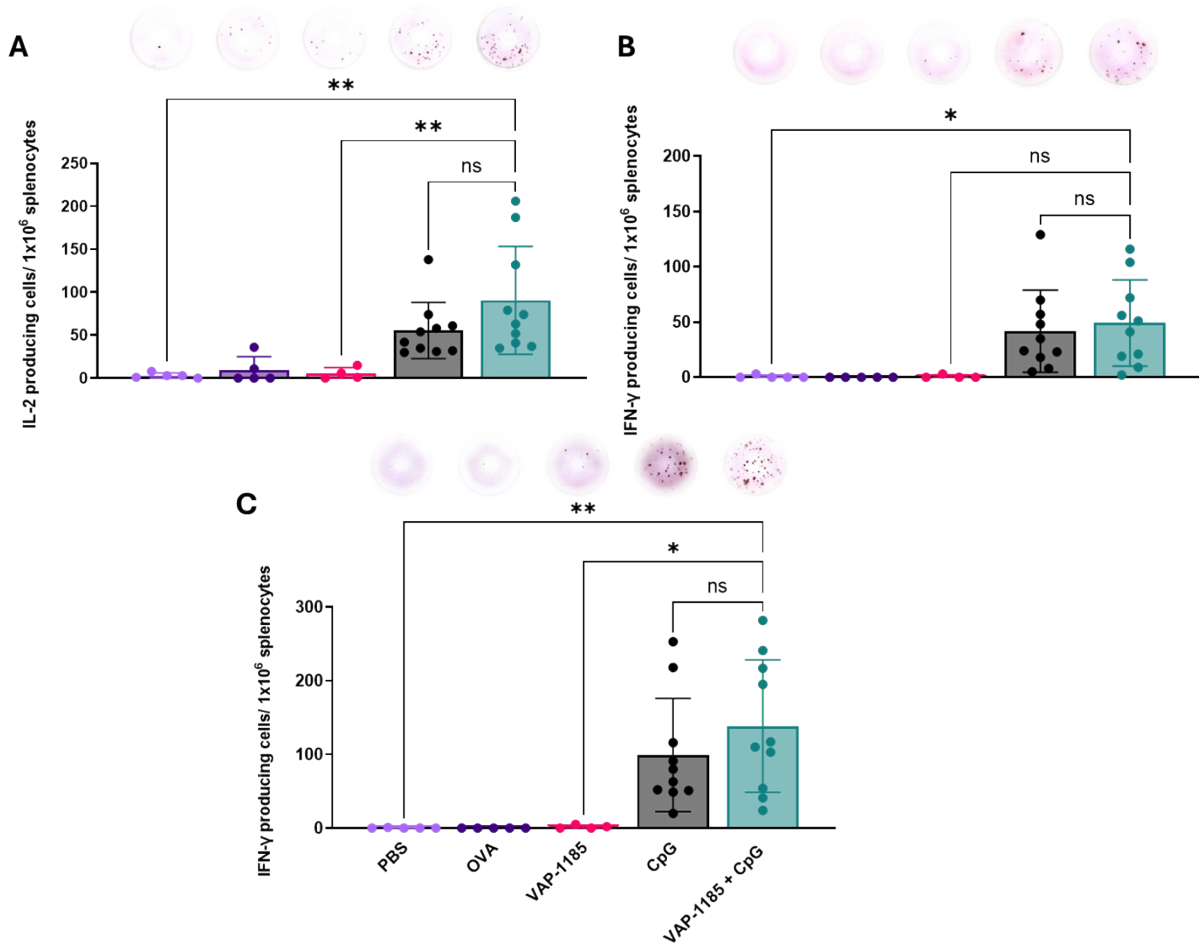
Supplemental Figure 3. Mass spectrum of VAP-1185. Predicted: $C_{19}H_{23}F_3N_4 [M+H]^+$: $m/z = 365.2$; Observed: $C_{19}H_{23}F_3N_4 [M+H]^+$ $m/z = 365.19472$. Mass error = -0.103 ppm.



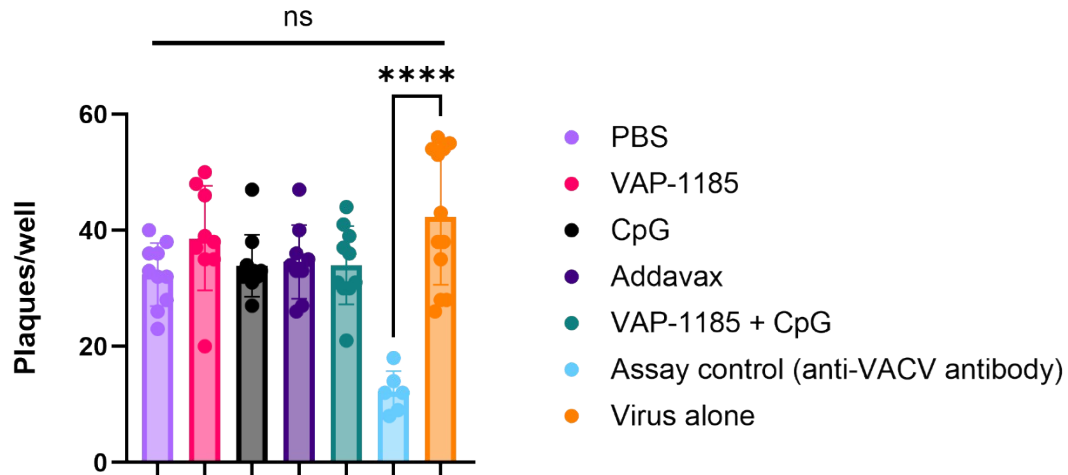
Supplemental Figure 4. RPMI 2650 cells were treated with VAP-1185 for 30 minutes and TEER was measured. Data presented as mean + SD.



Supplemental Figure 5. Mice were euthanized on day 63 and total IgG was analyzed for (A) NW and (B) BAL. Data presented as mean + SD. Significant differences determined by using a one-way ANOVA with Tukey's multiple comparisons (ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$).



Supplemental Figure 6. ELISpots of splenocytes stimulated with the complete OVA protein that produced (A) IL-2 and (B) IFN- γ . ELISpot of splenocytes stimulated with the OVA peptide, SIINFEKL that produced (C) IFN- γ . (For PBS, OVA, and VAP-1185 groups $n=5$; for CpG and VAP-1185+CpG groups $n=10$). Data presented as mean \pm SD. Significant differences determined by using a one-way ANOVA with Tukey's multiple comparisons (ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$).



Supplemental Figure 7. Serum from day 56 was used to perform a PRNT assay. Data presented as mean \pm SD. Significant differences determined by using a one-way ANOVA with Tukey's multiple comparisons (ns = not significant; **** $p \leq 0.001$).