

Synthetic Chimeric Horsepox Virus (scHPXV) Vaccination Protects Macaques from Monkeypox

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Introduction

Despite its eradication, smallpox remains a biothreat. While US military Global Response Force service troops are immunized with clonal, live vaccinia virus (VACV), safety concerns limit its further use in groups like first responders. There is a need for an effective but better tolerated, single dose, live replicating smallpox vaccine. Sequence analysis of polyclonal or old smallpox vaccines indicate a common ancestor with horsepox virus (HPXV)¹, and suggest that modern VACV diverged in the core viral sequence, and in the accumulation of deletions in the left and right inverted terminal repeats (ITRs). However, it is unknown if the early HPXV-like vaccines, which protected against smallpox, exhibited different safety and efficacy profiles compared to modern VACV. To assess the tolerability and vaccine activity of a synthetic/chimeric (sc)HPXV, four groups of macaques were vaccinated either with two different doses of scHPXV, one dose of synthetic VACV (sVACV), or vehicle prior to challenge with monkeypox virus (MPXV).

Figure 1. Phylogenetic relationships between VACV strains and HPXV

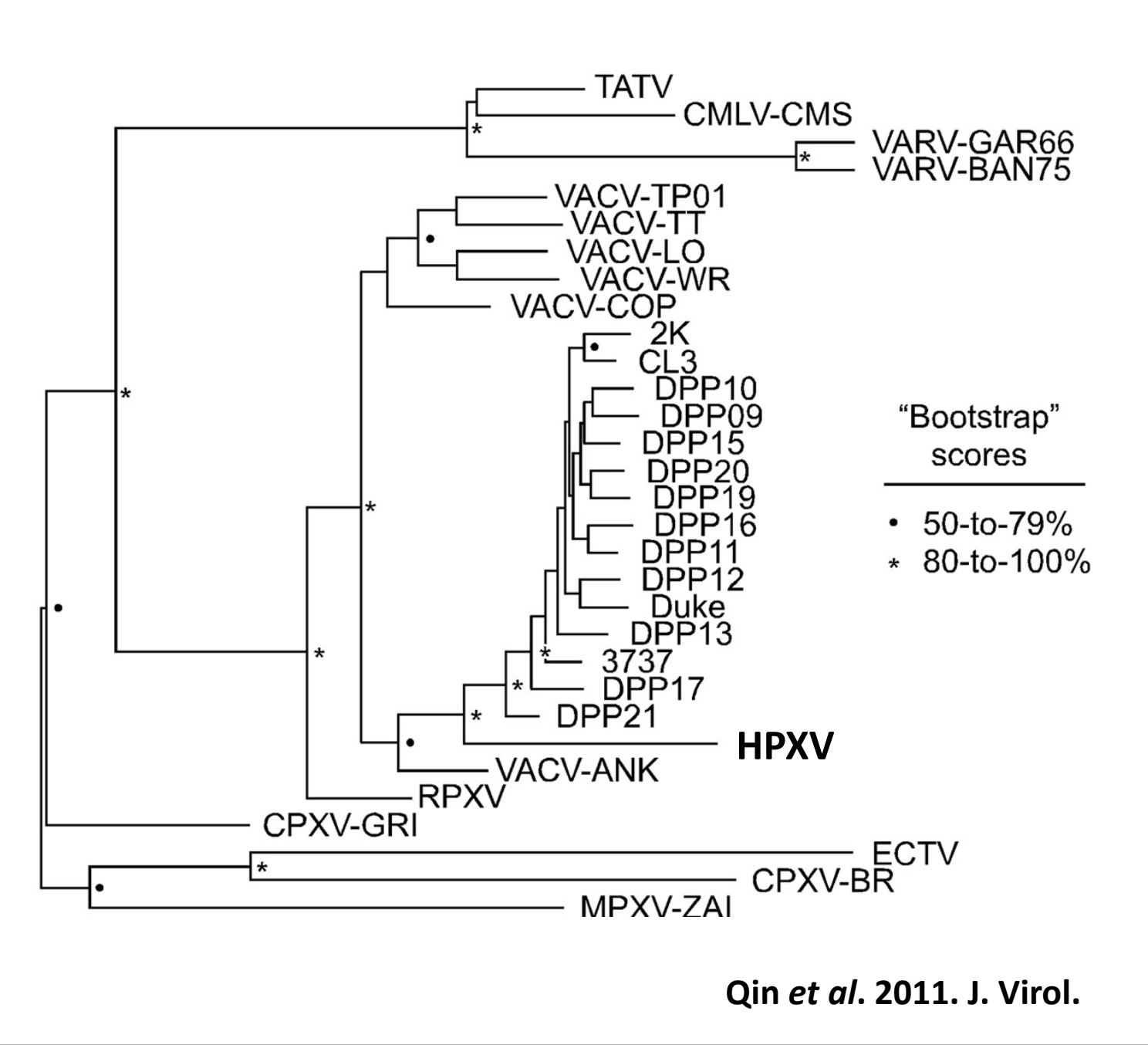
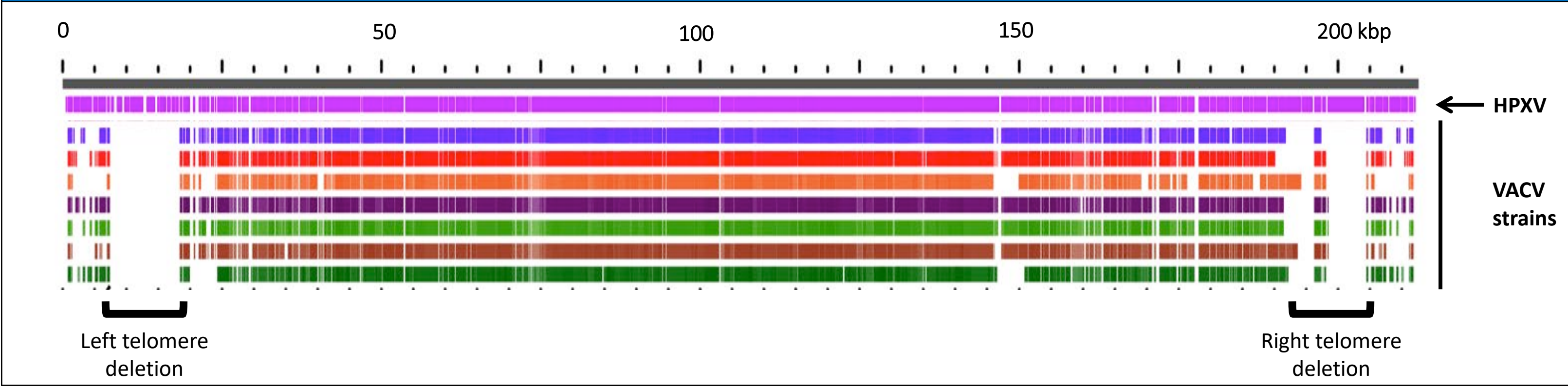


Figure 2. Similarities between VACV strains and HPXV:



Experimental Procedures

scHPXV and sVACV were assembled using synthetic DNA fragments as described previously². A laboratory isolate of VACV was sequenced using Illumina sequencing technologies to obtain a complete genome sequence, including the terminal hairpin sequences and repeat region in the inverted terminal repeat (ITR). This sequence is very similar to VACV (strain ACAM 2000) and has been deposited in Genbank (Accession # **MN974380**). The sVACV genome sequence was also deposited into Genbank (Accession # **MN974381**). Cynomolgus macaques (4 per group) were vaccinated *via* scarification using a bifurcated needle with 4x10⁶ or 5x10⁵ PFU of scHPXV, 8x10⁴ PFU of sVACV, or PBS, before challenge 60 days later with 9x10⁴ PFU of MPXV (strain Zaire) *via* the intratracheal (*i.t.*) route.

Table 1. Experimental Design for scHPXV Dose-Range Finding Study in NHP.

Group	N	Vaccine (Day 0)	Vaccination Dose (PFU)*	Route of Vaccine Administration	IT Challenge Dose (Day 60)
1	4	scHPXV	4x10 ⁶	Scarification	9x10 ⁴ PFU
2	4	scHPXV	5x10 ⁵	Scarification	9x10 ⁴ PFU
3	4	sVACV	8x10 ⁴	Scarification	9x10 ⁴ PFU
4	4	Vehicle	N/A	Scarification	9x10 ⁴ PFU

*A second vaccination by scarification (following procedures and dose volume used on day 0) was given to four animals that did not show evidence of a take at the vaccination site by day 7. The re-vaccination of these four animals occurred on day 14.

Figure 3. Treatment Timeline

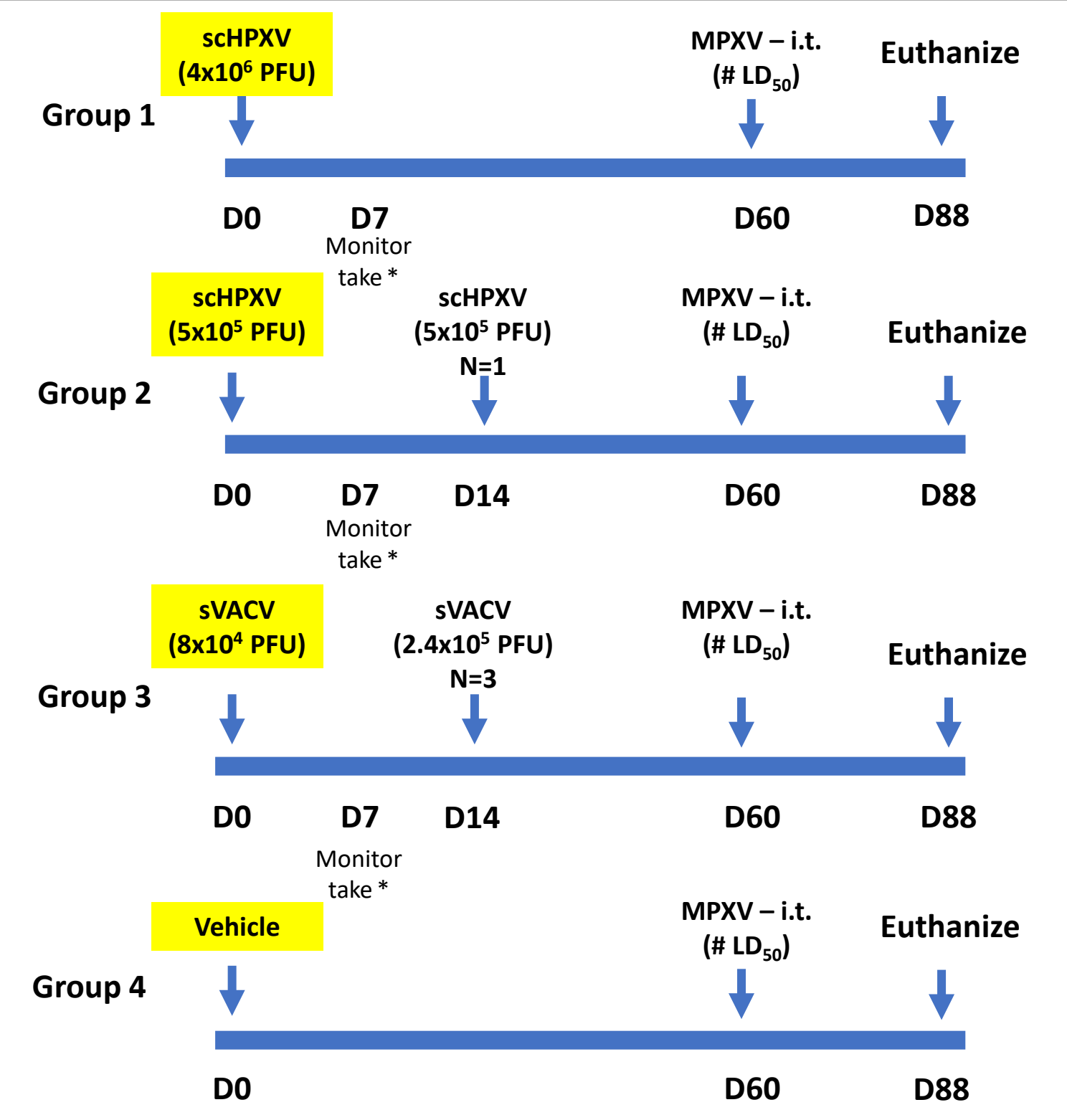
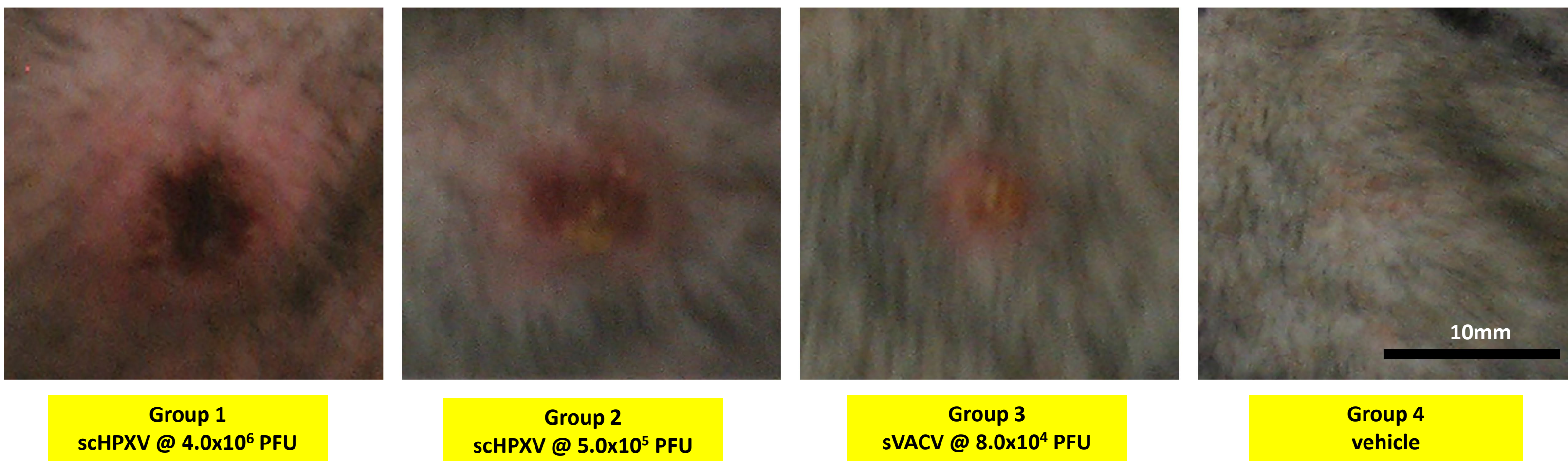


Figure 4. Representative Images of Vaccination Site on Day 7:



Results

Two different doses of scHPXV vaccine were tested: 4 of 4 animals in the 4x10⁶ PFU dose, and 3 of 4 animals in the 5x10⁵ PFU dose groups exhibited a “take” at day 7. A take is a biomarker of protective immunity in immunocompetent recipients receiving live VACV vaccine and has been successfully used in campaigns to prevent smallpox contagion. In the sVACV arm only 1 of 4 animals exhibited a take. The animals that did not present a take were revaccinated on day 14: the one scHPXV animal was revaccinated with 5x10⁵ PFU scHPXV and the 3 sVACV animals were revaccinated with 2.4x10⁵ PFU sVACV. All but one of the sVACV animals subsequently produced a take. After MPXV challenge no lesions were seen in any of the 8 animals vaccinated with scHPXV. One animal in the sVACV arm died from unrelated causes, but while the 3 remaining animals all had takes, 2 still showed lesions by day 69. Symptoms characteristic of systemic monkeypox infections were seen in all 4 vehicle animals by day 69.

scHPXV is Tolerated During Vaccination of Macaques

Figure 5. Weights of Macaques During Vaccination Period

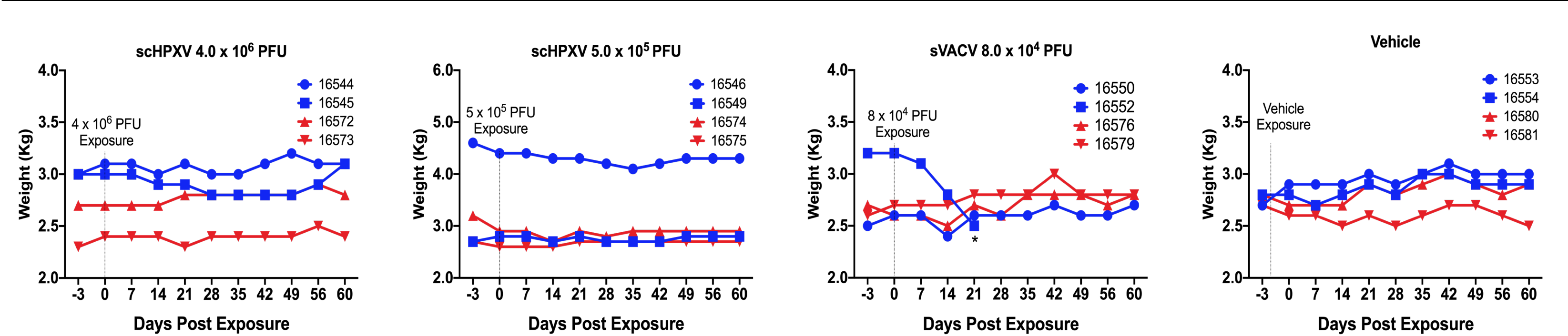
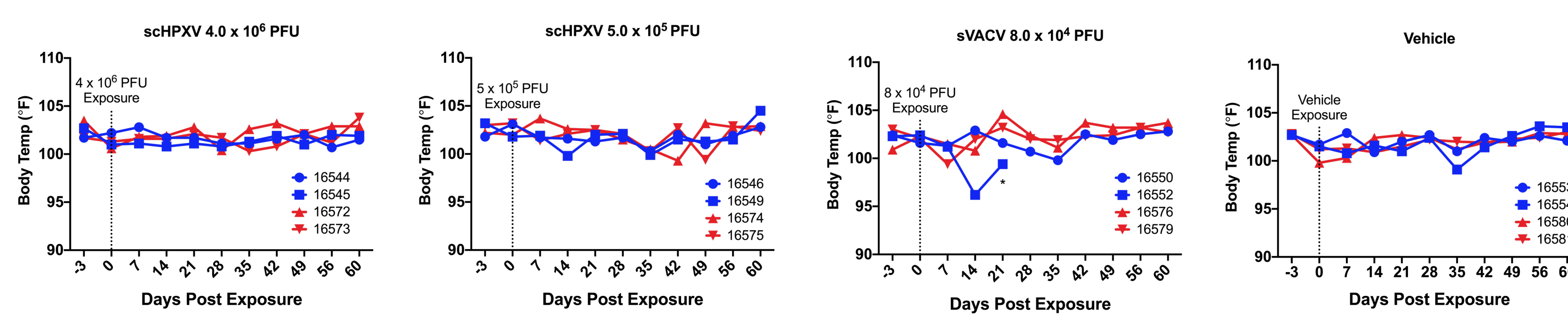


Figure 6. Body Temperature of Macaques During Vaccination Period



scHPXV Protects Macaques from Intratracheal MPXV Challenge

Figure 7. Weight Change

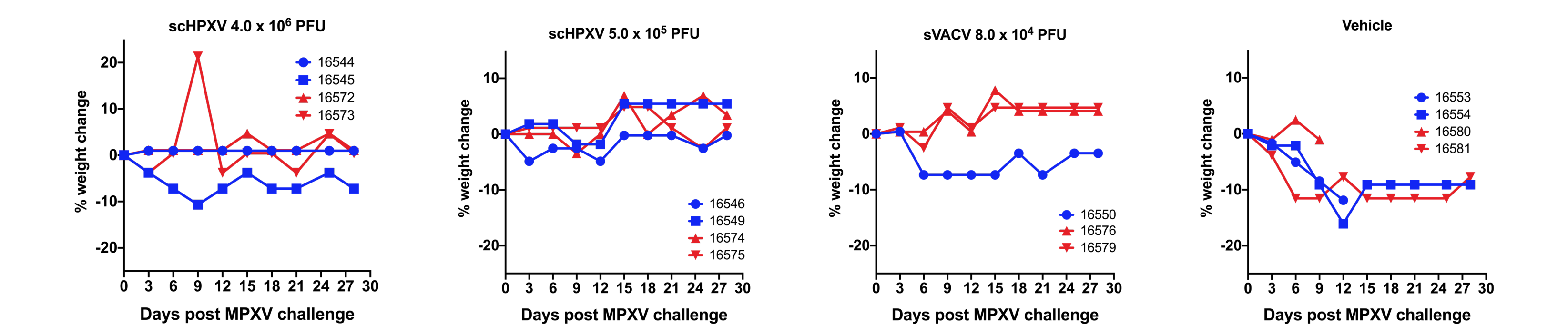


Figure 8. Change in Body Temperature

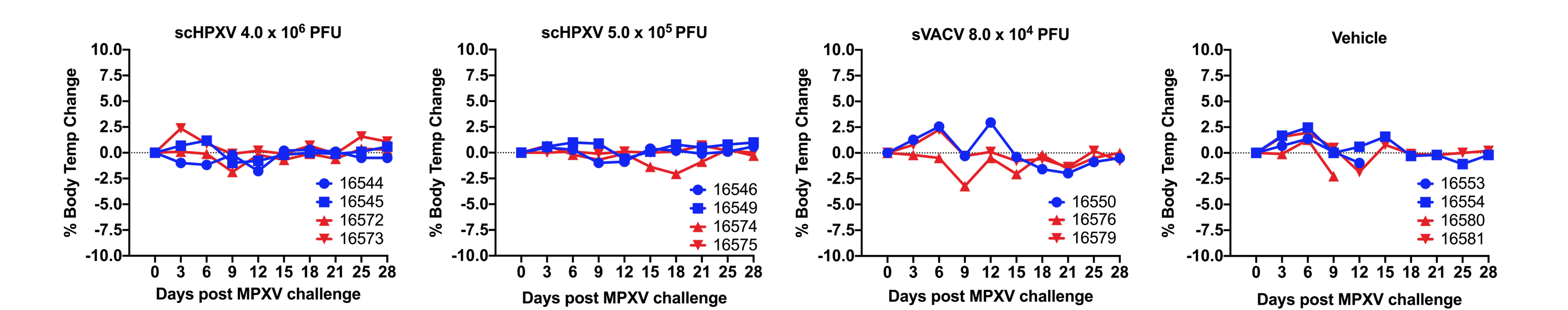
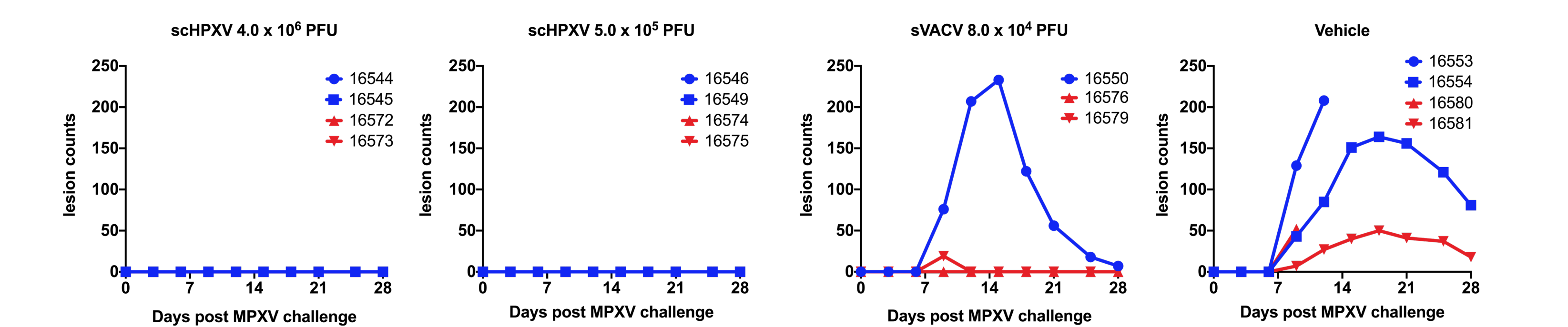


Figure 9. Lesion Counts



Conclusions

- HPXV virus is closely related to VACV
- Molecular evolution indicates that HPXV is a primordial strain (with “complete” left and right ITRs).
- HPXV has additional genes, relative to VACV that are mostly involved in host immune interactions
- HPXV likely closer to the vaccine used by Edward Jenner than more recent strains (in terms of left and right ITRs and core viral sequence).
- Tolerability of HPXV at low and high dose is comparable to VACV at low dose.
- Protection of HPXV and VACV was comparable (all 8 HPXV animals and all 3 VACV animals survived and recovered)
- HPXV had higher rates of “take” (4/4 high dose and 3/4 low dose) than VACV (1/3) after a single vaccination.
- “Take” is the major cutaneous reaction that is a biomarker of protective and sterilizing immunity in immunocompetent individuals vaccinated with a live vaccinia virus
- HPXV induced sterilizing immunity (no lesions) in all 8 animals (4 high dose and 4 low dose), while VACV (low dose) provided sterilizing immunity only 1 of 3.