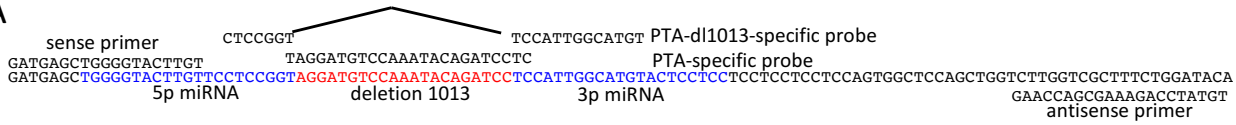
A



B

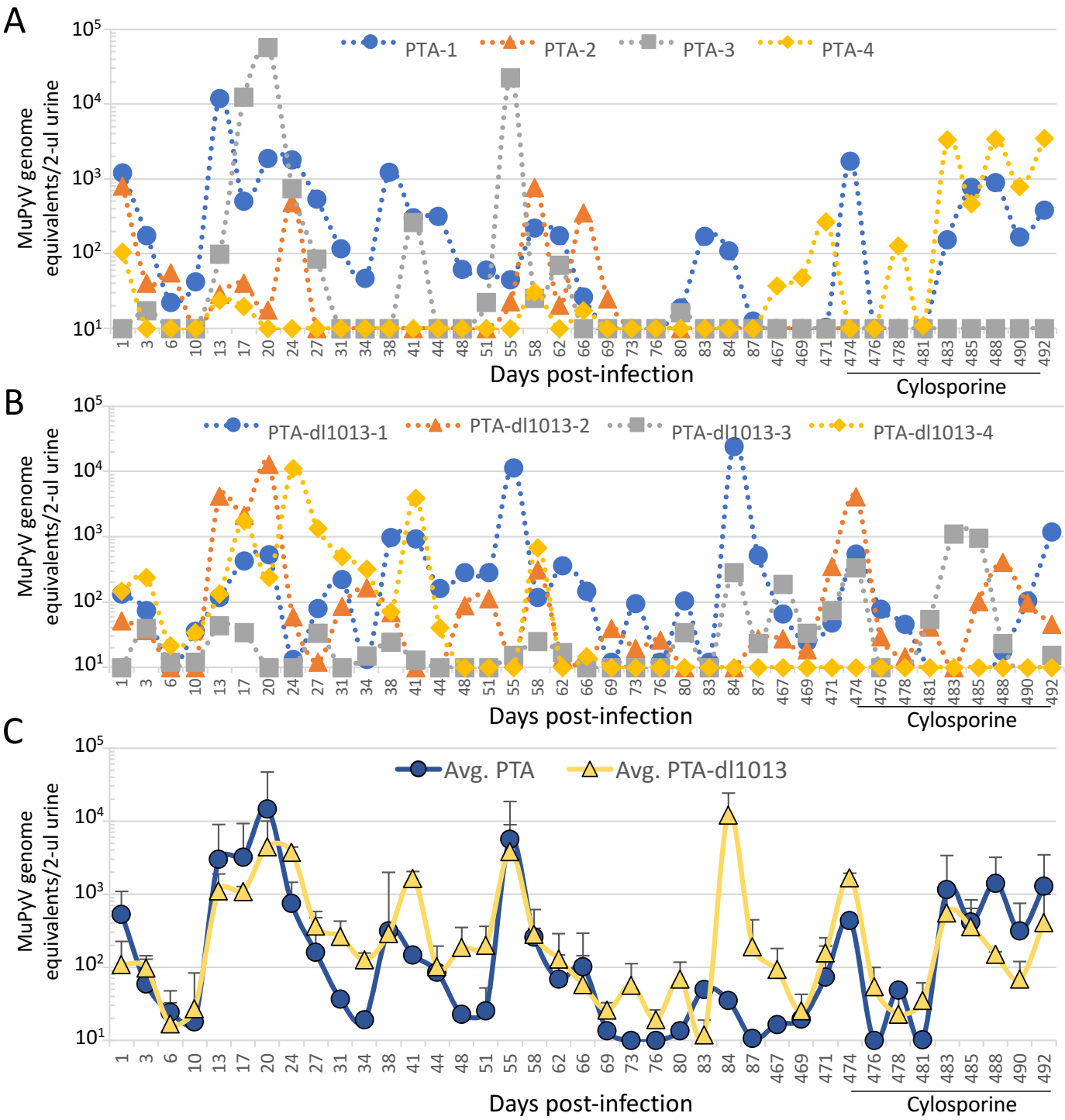
copy number	MuPyV target primers/probes			
	standard target	Ct	SD	copy number
10^7	PTA	12.9	0.04	10,000,000
	PTA-dl1013	12.6	0.04	13,194,764
10^6	PTA	16.3	0.08	1,000,000
	PTA-dl1013	16.0	0.48	1,378,592
10^5	PTA	19.8	0.08	100,000
	PTA-dl1013	19.9	0.37	96,534
10^4	PTA	23.4	0.08	10,000
	PTA-dl1013	23.3	0.03	8,882
10^3	PTA	26.5	0.87	1,000
	PTA-dl1013	27.1	0.42	712
10^2	PTA	30.3	0.35	100
	PTA-dl1013	29.7	0.27	119
10^1	PTA	32.9	0.21	10
	PTA-dl1013	33.2	0.42	11

C

		Target primers/probes			
		PTA		PTA-dl1013	
Standard	copy number	Ct Mean	Ct SD	Ct Mean	Ct SD
PTA	10^6	15.2	0.1	38.6	1.7
PTA-dl1013	10^6	n.d.	n.d.	16.3	0.4

Supplemental Figure 1. Specific detection and quantitation of PTA and PTA-dl1013 strains by PCR.

To distinguish between PTA and PTA-dl1013 genomes, we used custom TaqMan probes that either recognized PTA or PTA-dl1013. (A) Schematic diagram of the qPCR strategy to specifically recognize and quantitate the PTA and PTA-dl1013 genomes. The PTA-specific probe was designed to recognize the 21-bp sequence deleted in PTA-dl1013. The PTA-dl1013-specific probe was designed to recognize the sequences upstream and downstream of the 21-bp deletion in PTA-dl1013. The primers recognize the sequences in both PTA and PTA-dl1013 flanking the probe-binding location. (B) Genome copy number standards of PTA and PTA-dl1013 were made by serial dilution of purified plasmids containing the PTA or PTA-dl1013 genomes. qPCR analysis of PTA and PTA-dl1013 standards using the universal MuPyV primers/probe confirmed that the difference between the standards was less than one Ct value at each dilution. (C) qPCR analysis of the PTA and PTA-dl1013 10^6 copy number standards using the PTA and PTA-dl1013 specific probes demonstrated that PTA-dl1013 was undetected with the PTA probe, whereas PTA was largely undetected, or far less efficiently detected, with the PTA-dl1013 probe. This confirms that PTA and PTA-dl1013 probes specifically recognize their respective genomes. Samples in which threshold cycle (Ct) values were undetermined are marked n.d. The Ct values are the average of reactions run in triplicate, which were used to calculate the Ct value and standard deviation (SD).



Supplemental Figure 2. MuPyV shedding in urine of mice infected with either PTA or PTA-dl1013.

Four C57BL/6 female mice were infected with 10^5 IU of either PTA or PTA-dl1013, and DNA genome copies in 2 ul urine were quantified at various times p.i. qPCR analysis was performed using the universal primer/probes set that recognize both the PTA and PTAdl1013 genomes. Genome equivalents in individual mice infected with wt PTA are presented in Panel A; individual mice infected with PTA-dl1013 are in Panel B. Samples in which copy numbers were below the limit of detection (10 copies) were graphed at the limit of detection. Average genome copies (±SE) of PTA (circles) and PTA-dl1013 (triangles) are presented in Panel C.