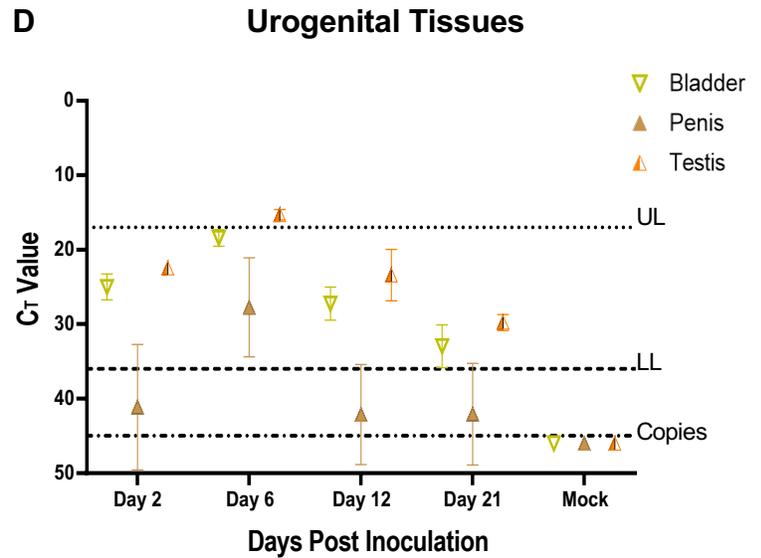
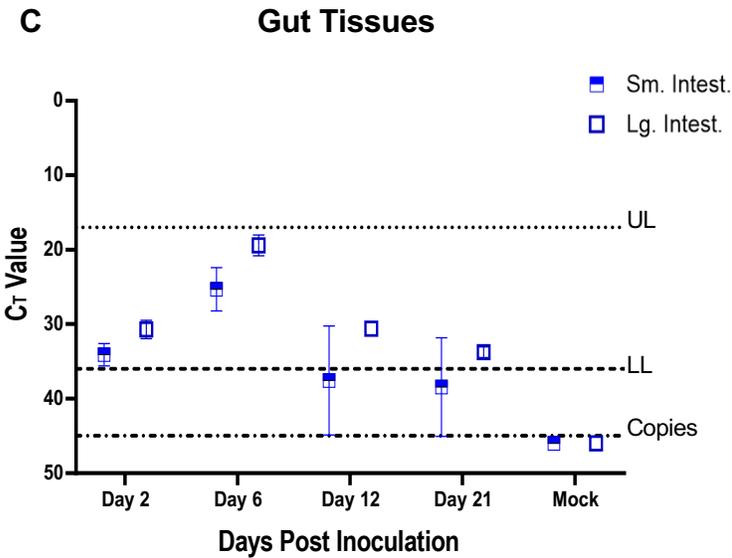
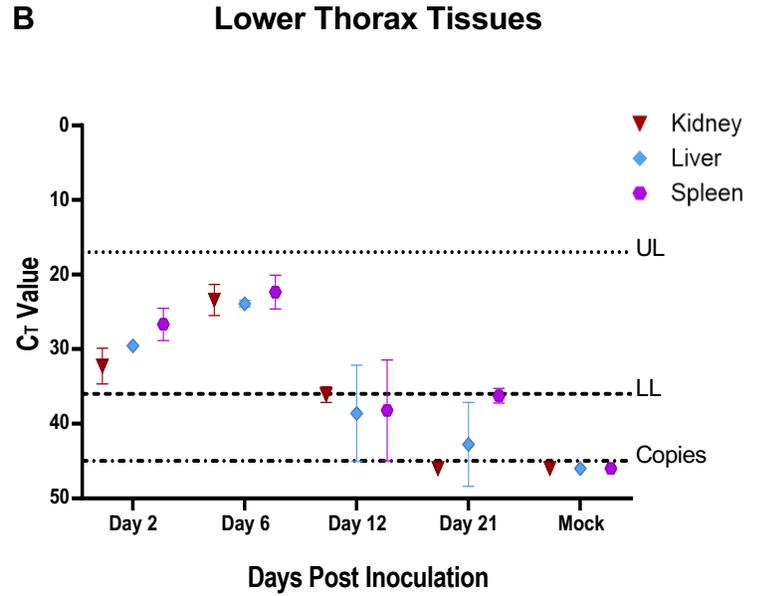
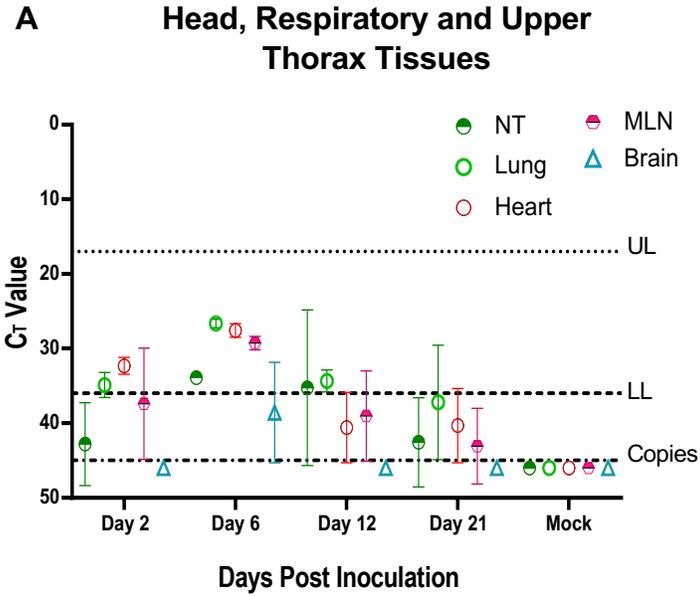


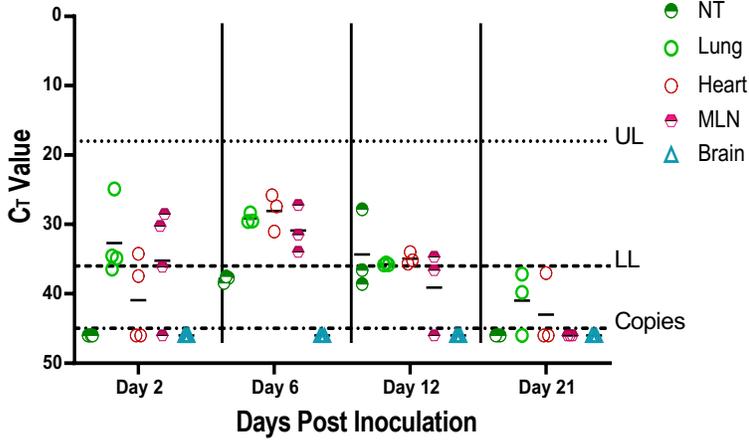
MPXV Clade IIb DNA in CAST/EiJ mice inoculated by IP with 10^5 PFU per mouse – C_T Means and Standard Deviation Over Time



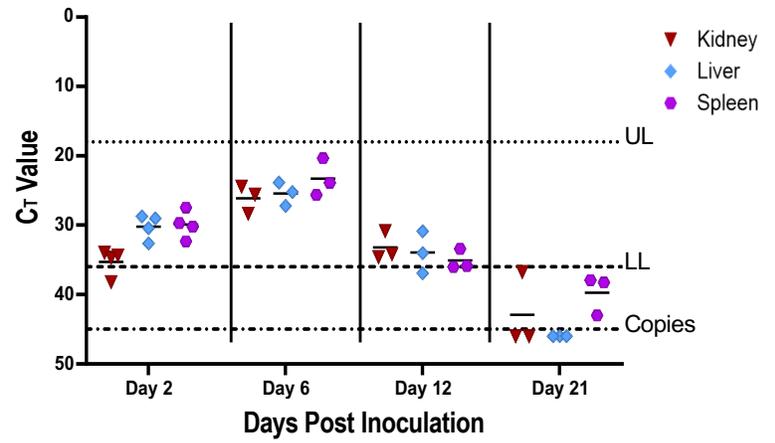
Supplementary Figure 1. Trends in viral DNA load over time in tissues from mice inoculated intraperitoneally with MPXV Clade IIb (1x10⁵ PFU per mouse). Male CAST/EiJ mice were inoculated with 1x10⁵ PFU of MPXV Clade IIb per animal through intraperitoneal (IP) injection. Tissues were collected at Day 2, 6, 12, and 21 post inoculation (n=3 for each time point). Taqman quantitative PCR was used to determine MPXV Clade IIb DNA levels in tissues from inoculated mice. Average C_T values ± SD were plotted in each graph to evaluate trends in tissue viral DNA over time. Tissue samples analyzed included nasal turbinate (NT), lung, heart, mediastinal lymph node (MLN), kidney, liver, spleen, small intestine (Sm. Intest.), large intestine (Lg. Intest.), brain, bladder, penis and testis. Data is grouped into functional regions of the body with the viral DNA detected in the head, respiratory and upper thorax shown in (A), the thorax in (B), the gut in (C) and in the urogenital tract shown in (D). The lower limit (LL) and upper limit (UP) of the assay range and total cycles used (45) are denoted by dashed lines. Upper and lower limits of the assay were determined from a standard curve of MPXV Clade IIb DNA. All tissues with an undetermined C_T value were assigned a value of 46 for purposes of graphing.

MPXV Clade IIb DNA in CAST/EiJ mice inoculated by IP with 10^4 PFU per mouse – C_T Means and Standard Deviation Over Time

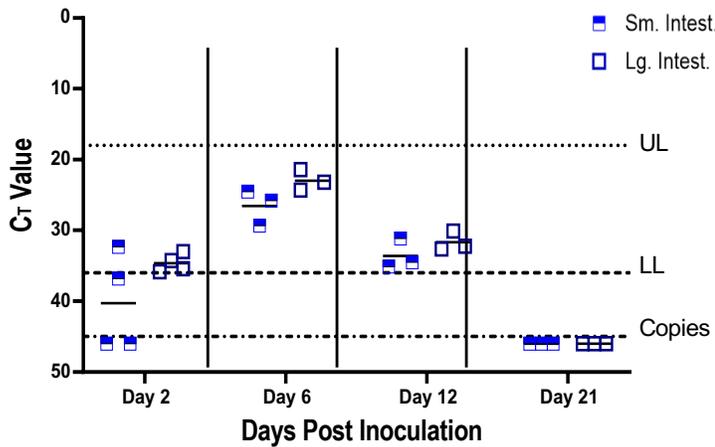
A Viral DNA in Head, Respiratory and Upper Thorax Tissues



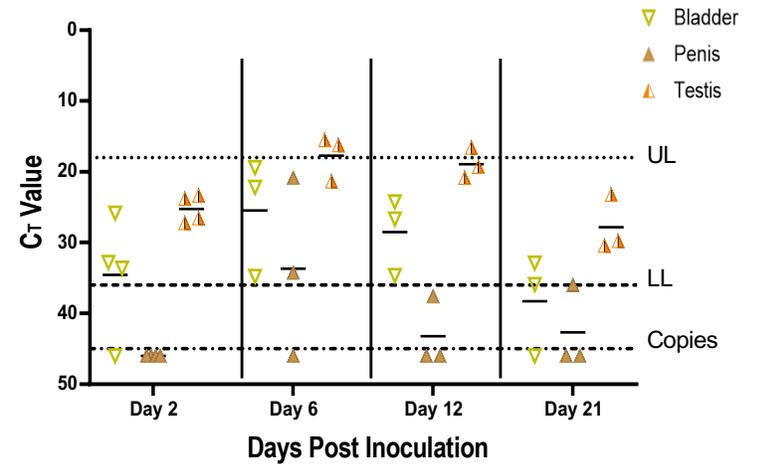
B Viral DNA in Lower Thorax Tissues



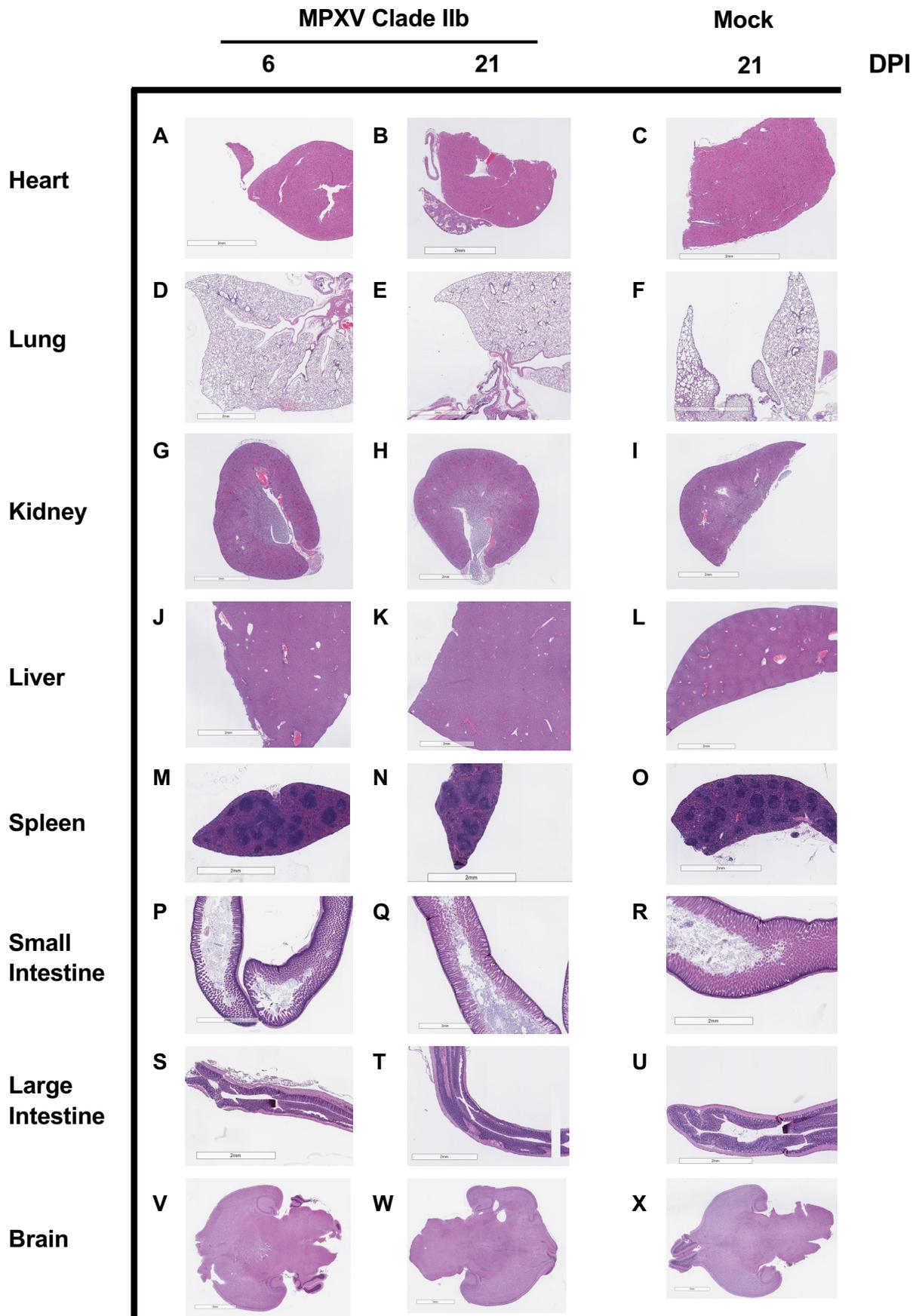
C Viral DNA in Gut Tissues



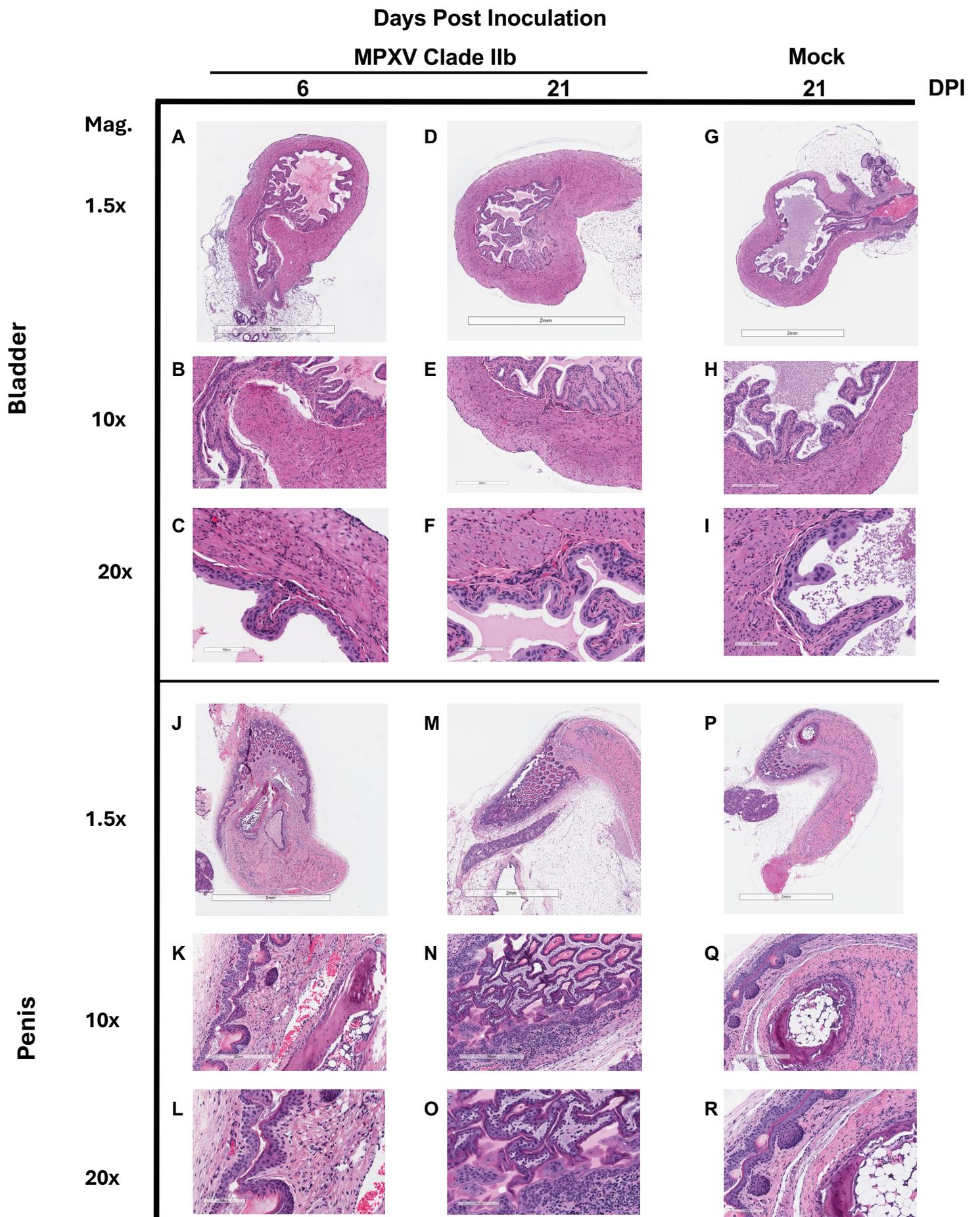
D Viral DNA in the Urogenital Tissues



Supplementary Figure 2. Intraperitoneal inoculation with 10^4 PFU MPXV Clade IIb per mouse results in systemic infection with no weight loss in CAST/EiJ mice. Male mice were inoculated with 1×10^4 PFU MPXV Clade IIb per animal through an intraperitoneal (IP) route of infection. Tissues samples were collected at Day 2, 6, 12, and 21 post inoculation (n=3 for each time point). Taqman quantitative PCR was used to determine MPXV Clade IIb DNA levels in tissues. The resulting critical threshold (C_T) values are shown in the graphs. Data is grouped into functional regions of the body with the viral DNA detected in the head, respiratory and upper thorax shown in (A), the thorax in (B), the gut in (C) and in the urogenital tract shown in (D). The lower limit (LL) and upper limit (UP) of the assay range and total cycles used (45) are denoted by dashed lines. Upper and lower limits of the assay were determined from a standard curve of MPXV Clade IIb DNA. All tissues with an undetermined C_T value were assigned a value of 46 for the purpose of graphing the data.

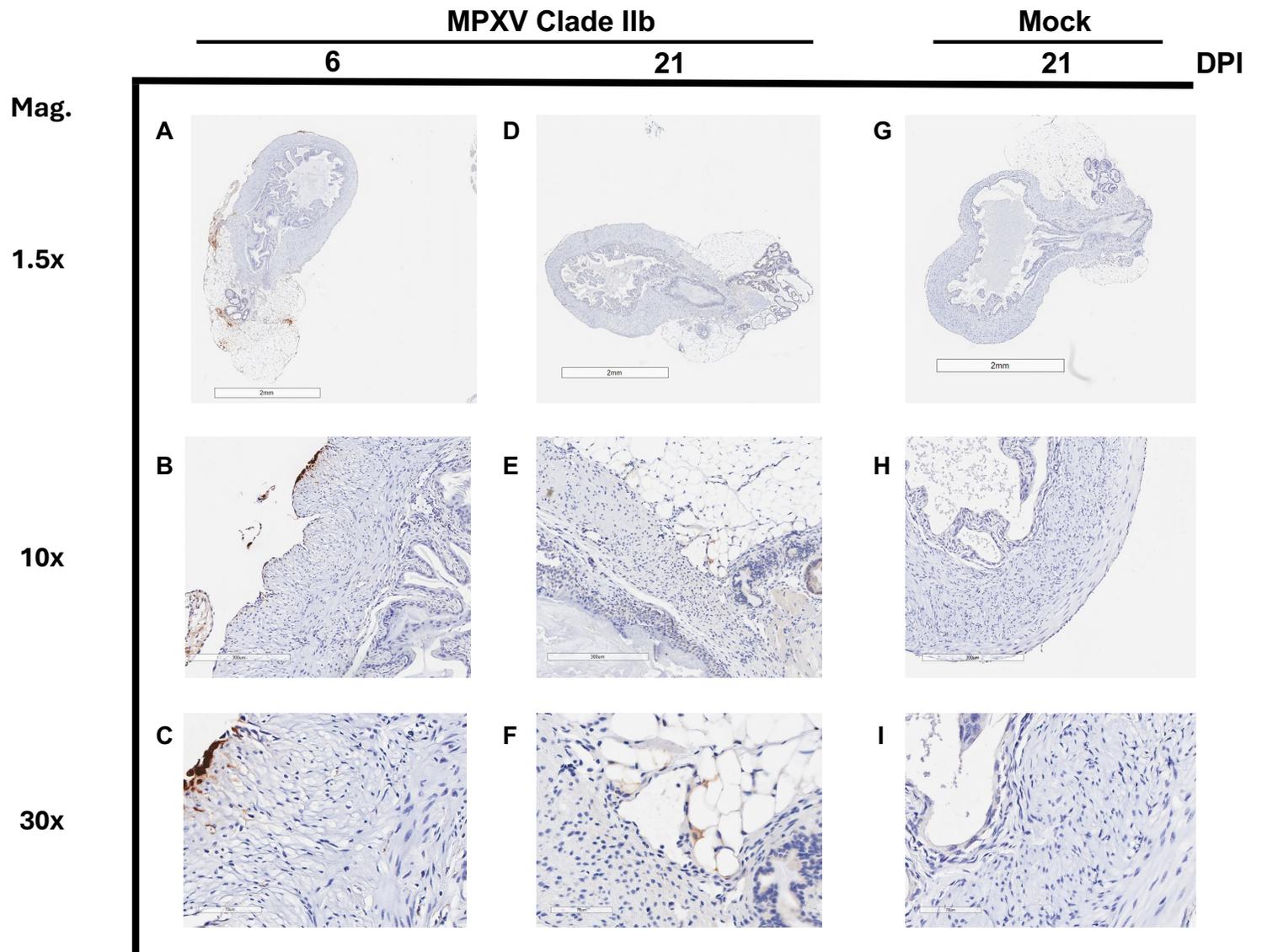


Supplementary Figure 3. Histological comparison of tissues from CAST/EiJ mice inoculated intraperitoneally with MPXV Clade IIb. Male mice were inoculated intraperitoneally (IP) with 1×10^5 PFU MPXV per animal. Mock animals were inoculated with 200 μ L of media intraperitoneally. Tissues were collected at 6 (n=3) and 21 days (n=3) post inoculation for MPXV Clade IIb inoculated animals and at 21 days post inoculation for the mock inoculated animals (n=3). Tissue samples were fixed in 10% formalin, paraffin embedded, mounted, and stained with a Hematoxylin and Eosin stain. Slides were analyzed by a board-certified pathologist. Representative tissue sections (n=3) from heart (A, B, C), lung (D, E, F), kidney (G, H, I), liver (J, K, L), spleen (M, N, O), small intestine (P, Q, R), large intestine (S, T, U), and brain (V, W, X) were compared between MPXV Clade IIb and mock infected animals. All images of tissues are shown at a 1.5x magnification. Scale bars, 2 mm for all tissues except brain. Scale bars for brain, 3 mm.



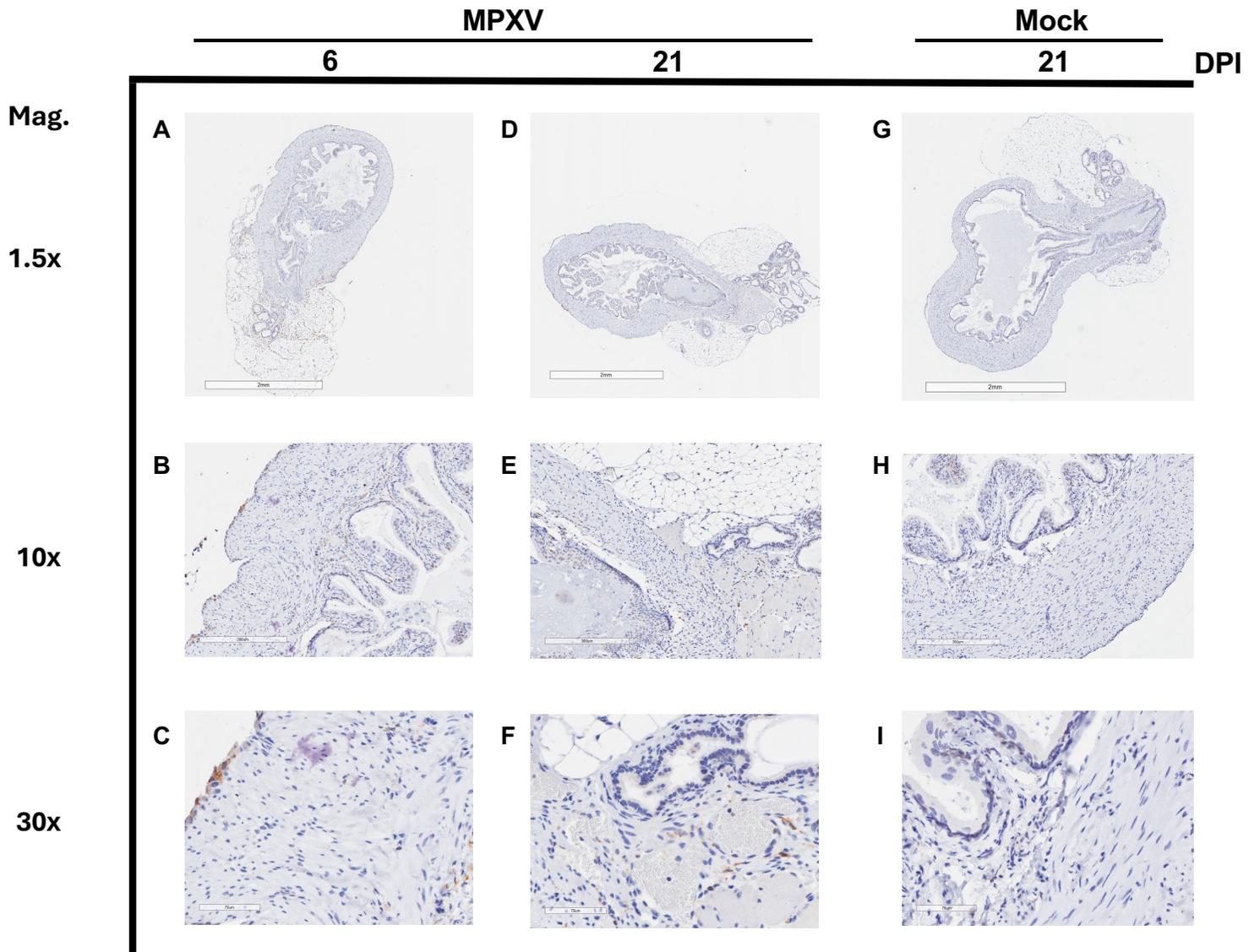
Supplementary Figure 4. Histological analysis in CAST/EiJ mouse bladder and penis after intraperitoneal inoculation with MPXV Clade IIb. Male mice were inoculated intraperitoneally with 1×10^5 PFU MPXV Clade IIb per animal as previously described. Tissues were collected at specified time points and then fixed in 10% formalin, paraffin embedded, mounted and stained with a Hematoxylin and Eosin stain. Slides were analyzed by a board-certified pathologist. Representative tissue sections from bladder (n=3) are shown at 1.5x (A, D, G), 10x (B, E, H) and at 20x (C, F, I). Representative tissues from penis (n=3) are shown at 1.5x (J, M, P), 10x (K, N, Q) and at 20x (L, O, R) magnification. Scale bars, 2 mm for figures at 1.5x magnification, 300 μ m for 10x magnification and 100 μ m for 20x magnification.

Bladder – Vaccinia Viral Antigen IHC



Supplementary Figure 5. Poxvirus antigen detected in bladder of CAST/EiJ mice Day 6 and 21 after IP inoculation with MPXV Clade IIb. Male mice were inoculated interperitoneally with 1×10^5 PFU MPXV Clade IIb per animal. Mock animals were inoculated with 200 μ L of media intraperitoneally. Tissue samples were fixed in 10% formalin and paraffin embedded. Viral antigen (brown) was detected in bladder by IHC using an anti-Vaccinia virus antibody (abcam 35219). Representative tissue sections of bladder from Day 6 post inoculation (A, B, C; n=3), 21 post inoculation (D, E, F; n=2) and mock infected mice (G, H, I; n=3) are shown. Scale bars at 2 mm for images at 1.5x magnification, 300 μ m for 10x magnification and 70 μ m for 30x magnification. DPI = day post inoculation

Bladder – IBA1 IHC



Supplementary Figure 6. Macrophage IBA1 antigen detected in bladder of CAST/EiJ mice Day 6 and 21 after IP inoculation with MPXV Clade IIb. Male mice were inoculated interperitoneally with 1×10^5 PFU MPXV Clade IIb per animal. Mock animals were inoculated with 200 μ L of media intraperitoneally. Tissue samples were fixed in 10% formalin and paraffin embedded. IBA1 antigen (brown) was detected in testes by IHC using rabbit anti-IBA1 antibody (ThermoFisher PA5-27436). Representative tissue sections of bladder from Day 6 days post inoculation (A, B, C; n=3), 21 days post inoculation (D, E, F; n=2) and mock infected mice (G, H, I; n=3) are shown. Scale bars at 2 mm for images at 1.5x magnification, 300 μ m for 10x magnification and 70 μ m for 30x magnification.