

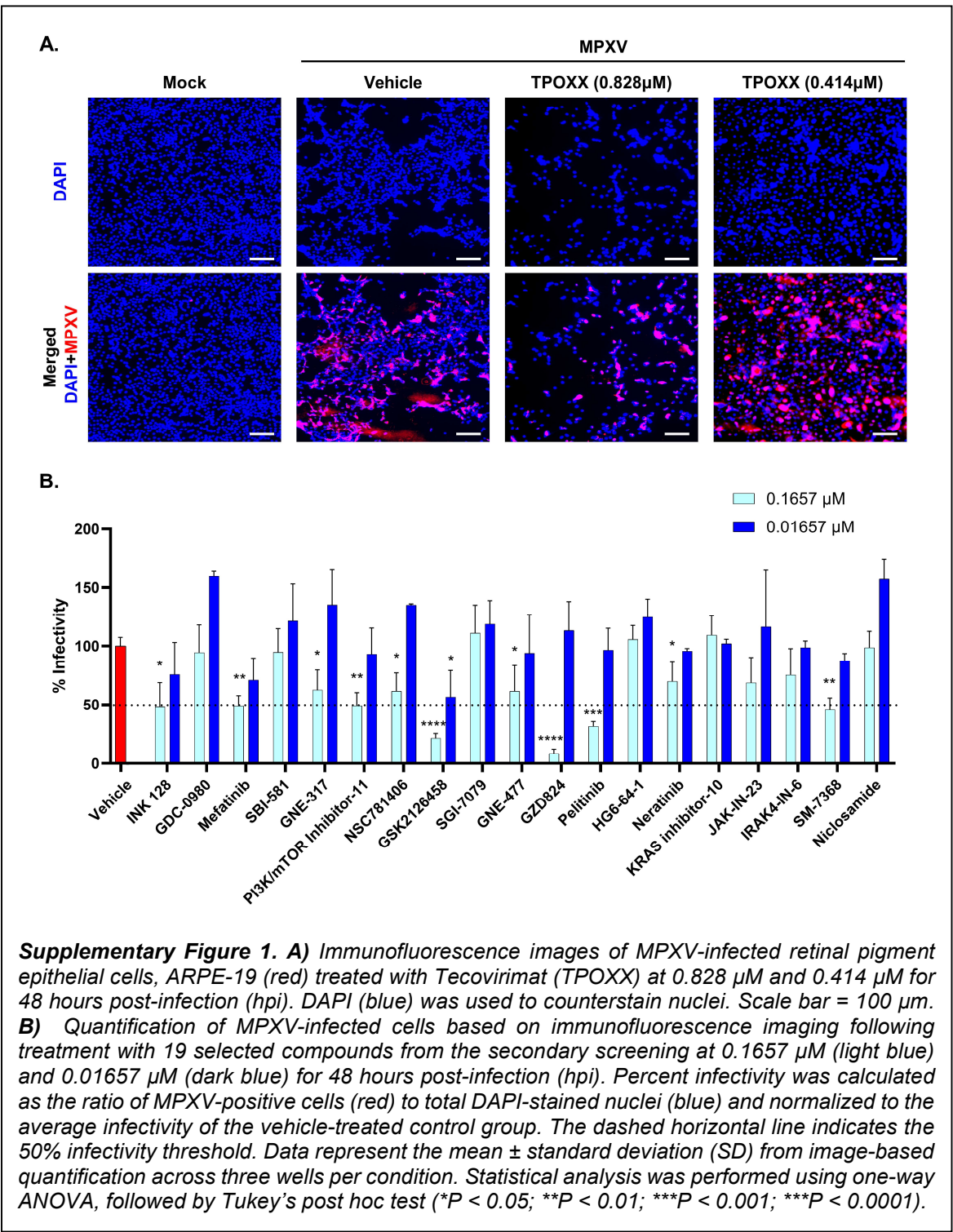
SUPPLEMENTAL FILE

Drug screen reveals new potent host-targeted antivirals against Mpox virus

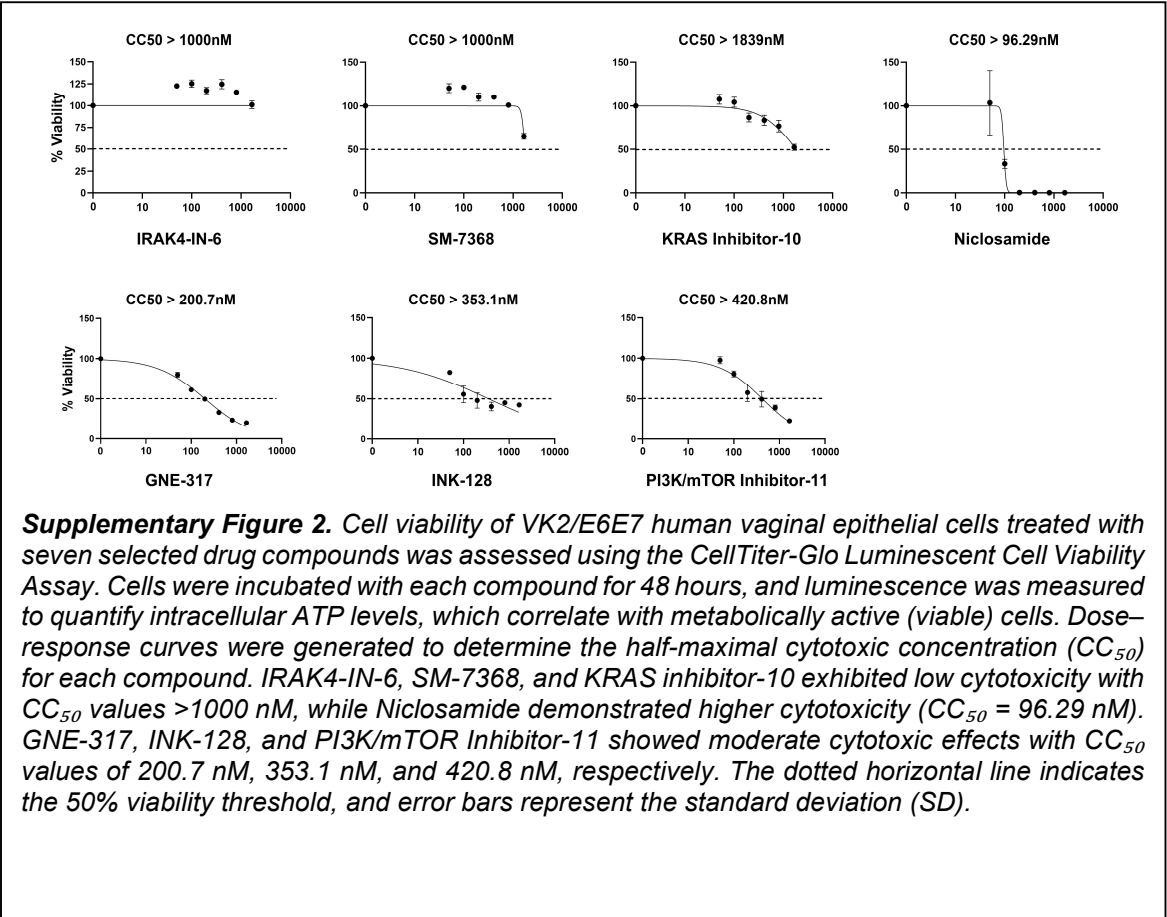
Arjit Vijey Jeyachandran, Anne K. Zaiss, Nikhil Chakravarty, Sneha Singh, Yennifer Delgado, Ramya Paravastu, Nivedha Satheeshkumar, Ephrem Gerald, Aakash Jeysankar, Joshua Thomas, Lilly Fuller, Noella Lee, Cameron Taylor, Shantanu Joshi, Mark Parcells, Samuel W. French, Abhijit Date, Mehdi Bouhaddou, Gustavo Garcia Jr, Ashok Kumar, Robert Damoiseaux, Vaithilingaraja Arumugaswami

SUPPLEMENTAL FIGURES AND LEGENDS

Supplementary Figure 1



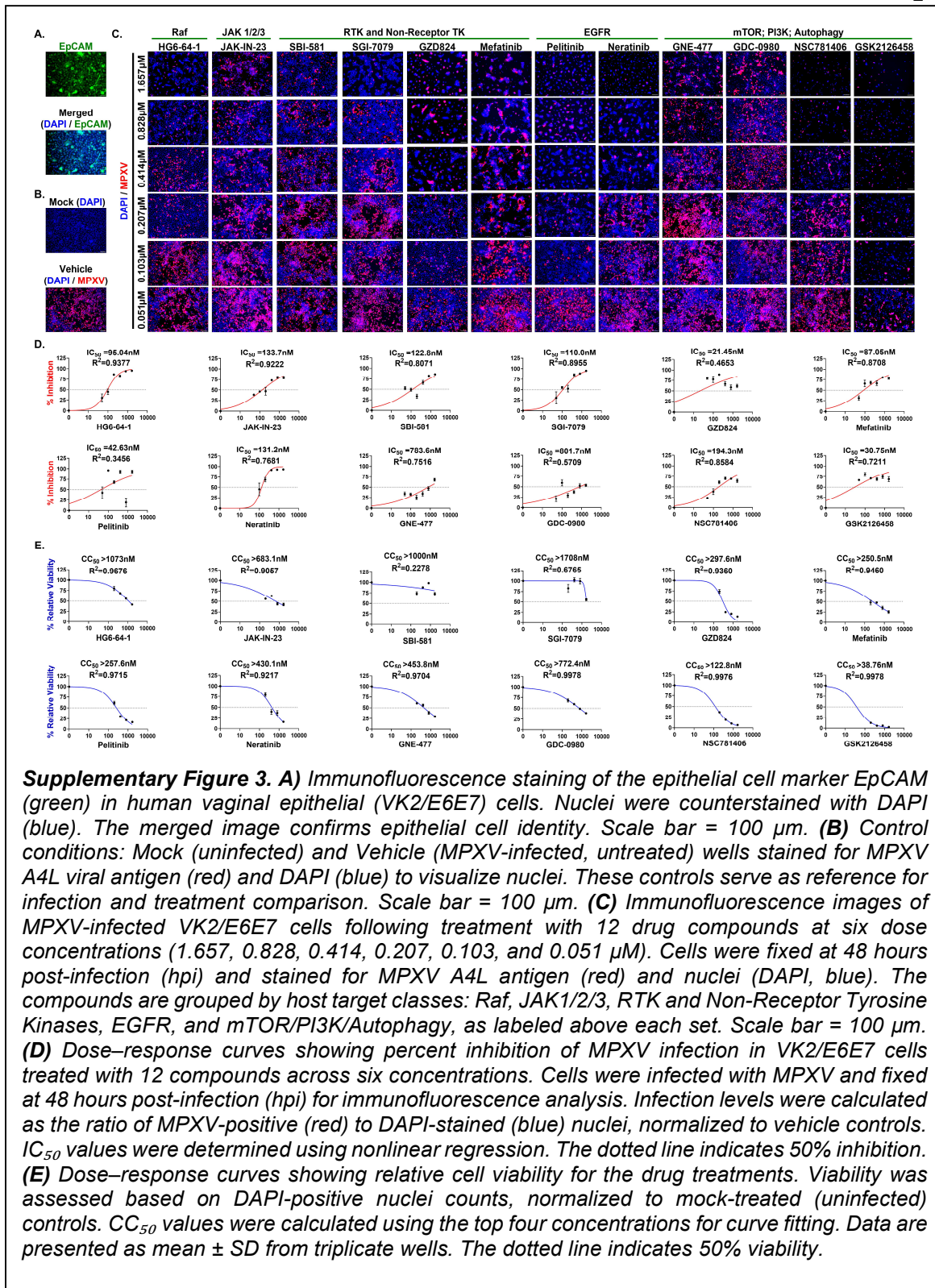
1 **Supplementary Figure 2**



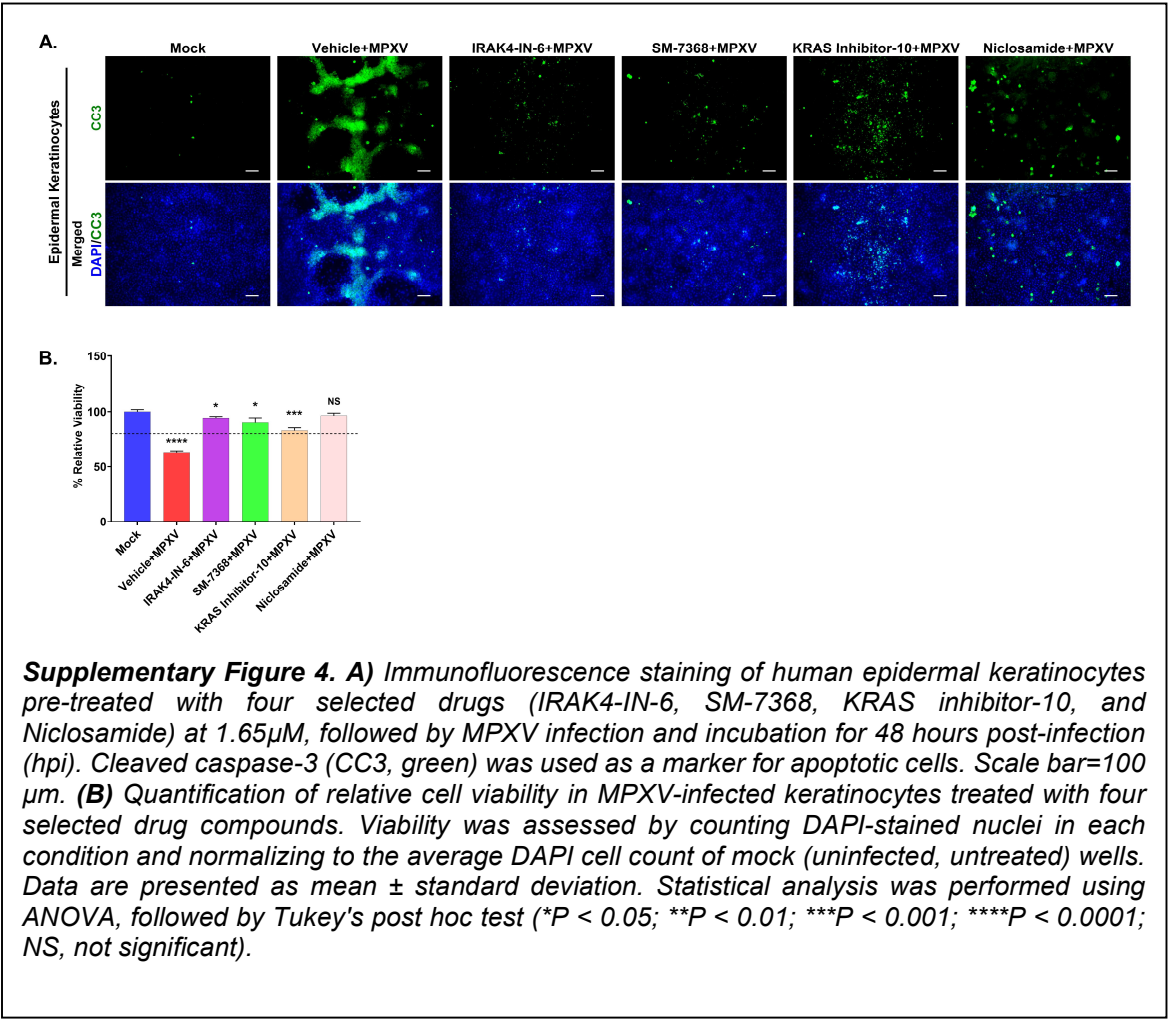
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1 Supplementary Figure 3

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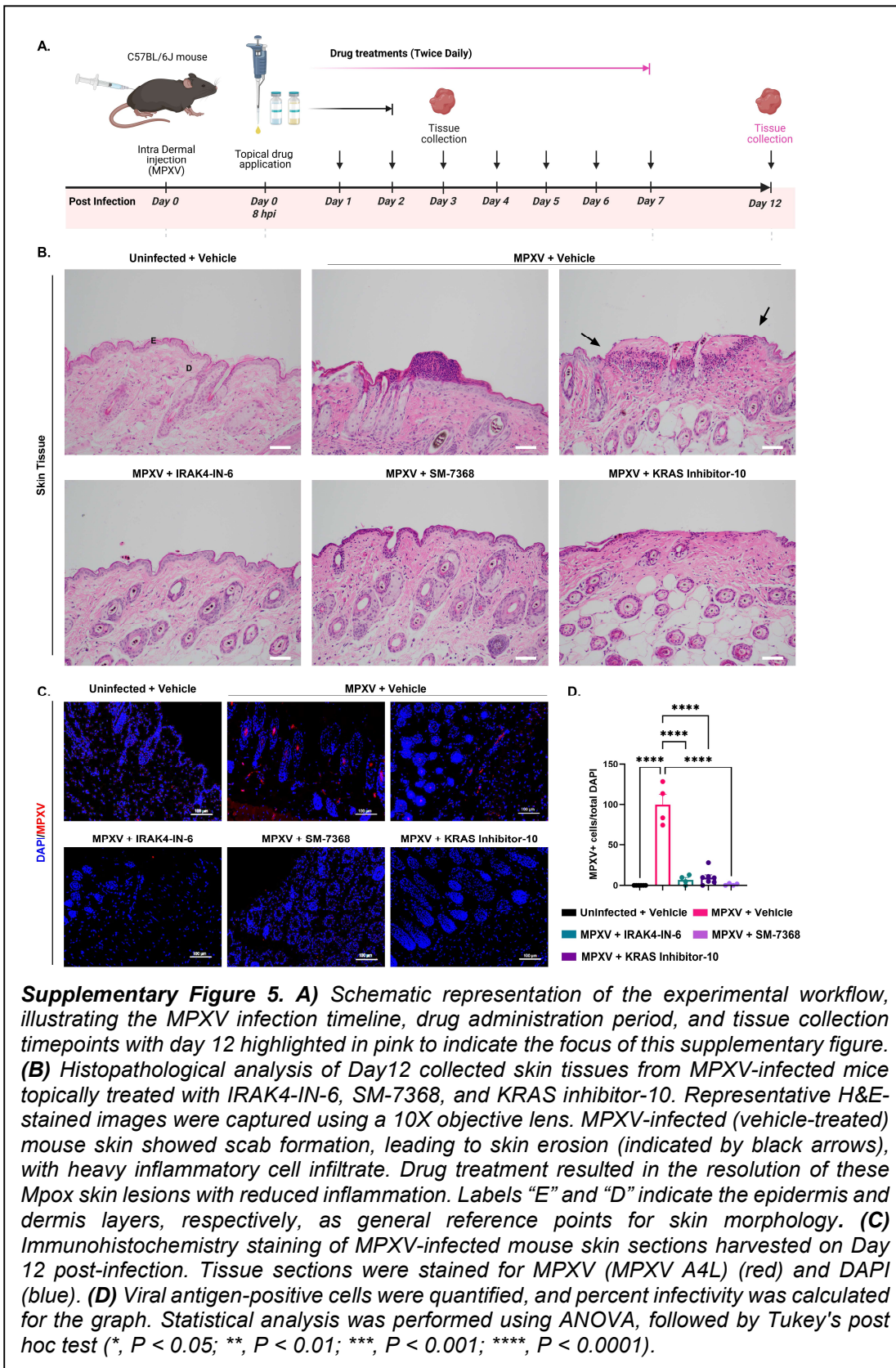


Supplementary Figure 4



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1 Supplementary Figure 5

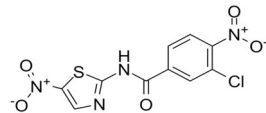
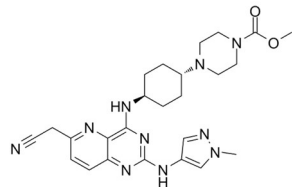
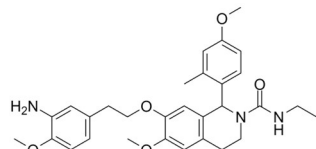


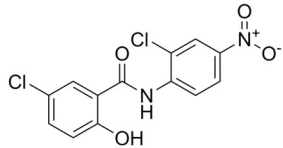
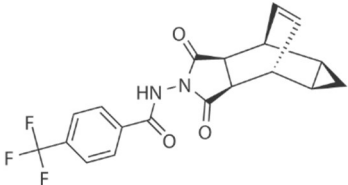
1 SUPPLEMENTARY TABLES

2 **Supplementary Table 1.** This table includes all 138 drug compounds identified in the primary
3 screening, along with their target pathways and molecular structures.

4 **Supplementary Table 2.**

| REAGENT/RESOURCE | SOURCE | IDENTIFIER |
|--|------------------------------|---------------|
| Antibodies | | |
| Mpox virus A4L antibody [HL2555] | GeneTex | Cat#GTX638927 |
| Phospho-NF- κ B p65 (Ser536) (93H1) Rabbit mAb | Cell Signaling | Cat#3033 |
| NF- κ B p65 (D14E12) XP® Rabbit mAb | Cell Signaling | Cat#8242 |
| Phospho-STING (Ser366) (E9A9K) Rabbit mAb | Cell Signaling | Cat#50907 |
| STING (D2P2F) Rabbit mAb | Cell Signaling | Cat#13647 |
| EpCAM (D9S3P) Rabbit mAb #14452 | Cell Signaling | Cat#14452 |
| Cleaved Caspase-3 (Asp175) Antibody #9661 | Cell Signaling | Cat#9661 |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 | Thermo Fisher Scientific | Cat#A-21422 |
| Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 | Thermo Fisher Scientific | Cat#A-11008 |
| Monoclonal Anti-Beta-Actin, Clone AC-74 produced in mouse | MilliporeSigma | Cat#A2228 |
| Bacterial and Virus Strains | | |
| hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb) | BEI Resources | Cat#NR-58622 |
| Chemicals, Peptides, and Recombinant Proteins | | |
| Regular Fetal Bovine Serum | Corning | Cat#35010CV |
| Eagle's Minimum Essential Medium (MEM) | Corning | Cat#10009CV |
| Penicillin-Streptomycin (10,000 U/mL) | Gibco | Cat#15140122 |
| L-Glutamine (200 mM) | Gibco | Cat#25030081 |
| MEM Non-Essential Amino Acids Solution(100X) | Gibco | Cat#11140050 |
| KGM™ Gold Keratinocyte Growth Medium BulletKit™ | Lonza | Cat#00192060 |
| DMEM/F-12, HEPES | Thermo Fisher Scientific | Cat#11330032 |
| SM-7368 | MedChemExpress | Cat#HY-116626 |
| IRAK4-IN-6 | MedChemExpress | Cat#HY-130253 |
| KRAS inhibitor-10 | MedChemExpress | Cat#HY-138295 |
| Tecovirimat | Selleckchem | CAT#S3380 |
| Methanol, Optima™ LC/MS Grade, Fisher Chemical | Fisher Scientific | Cat#A456-500 |
| Dimethyl sulfoxide | MilliporeSigma | Cat#D2650 |
| 16% Paraformaldehyde (formaldehyde) aqueous solution | Electron Microscopy Sciences | Cat#15710 |
| Dulbecco's Phosphate-Buffered Salt Solution 1X | Corning | Cat#21030CV |
| DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) | Thermo Fisher Scientific | Cat#D1306 |
| Corning™ Cell Culture Phosphate Buffered Saline (1X) | Thermo Fisher Scientific | Cat#MT21040CV |

| Bovine Serum Albumin | | MilliporeSigma | Cat#A9418 |
|--|--|------------------------|---|
| Normal Donkey Serum | | Jackson ImmunoResearch | Cat#017-000-121 |
| Normal Goat Serum | | Cell Signaling | Cat#5425S |
| Triton-X 100 | | MilliporeSigma | Cat#T9284 |
| Commercial Assays | | | |
| CellTiter-Glo Luminescent Cell Viability Assay | | Promega | Cat#G7570 |
| Experimental Models: Cell Lines | | | |
| VERO C1008 [Vero 76, clone E6, Vero E6] | | ATCC | Cat#CRL-158 |
| ARPE-19 | | ATCC | Cat#CRL-2302 |
| VK2/E6E7 | | ATCC | Cat#CRL-2616 |
| NHEK-Neo – Human Epidermal Keratinocytes, Neonatal, Pooled | | Lonza | Cat#00192906 |
| Software and Algorithms | | | |
| GraphPad Prism 10 | | GraphPad | N/A |
| Multi-Point Tool (Cell Counter) | | ImageJ | N/A |
| BioRender | | BioRender | N/A |
| | | | |
| COMPOUND DETAILS | MOLECULAR FORMULA/SEQUENCE | CAS# | STRUCTURE |
| SM-7368 | C ₂₅ H ₂₂ O ₁₀ | 380623-76-7 |  |
| IRAK4-IN-6 | C ₂₅ H ₃₂ N ₁₀ O ₂ | 2454244-02-9 |  |
| KRAS inhibitor-10 | C ₃₀ H ₃₇ N ₃ O ₅ | 2578876-75-0 |  |

| | | | |
|-------------|-------------------------|-------------|---|
| Niclosamide | $C_{13}H_8Cl_2N_2O_4$ | 50-65-7 |  |
| Tecovirimat | $C_{19}H_{15}F_3N_2O_3$ | 869572-92-9 |  |

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