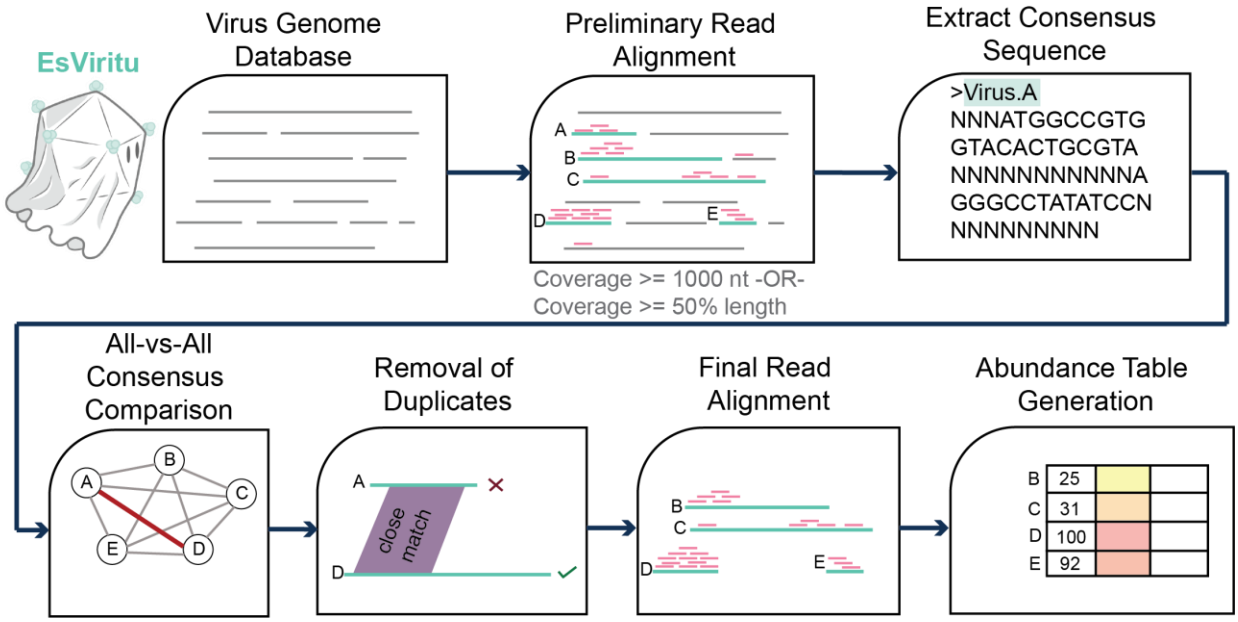
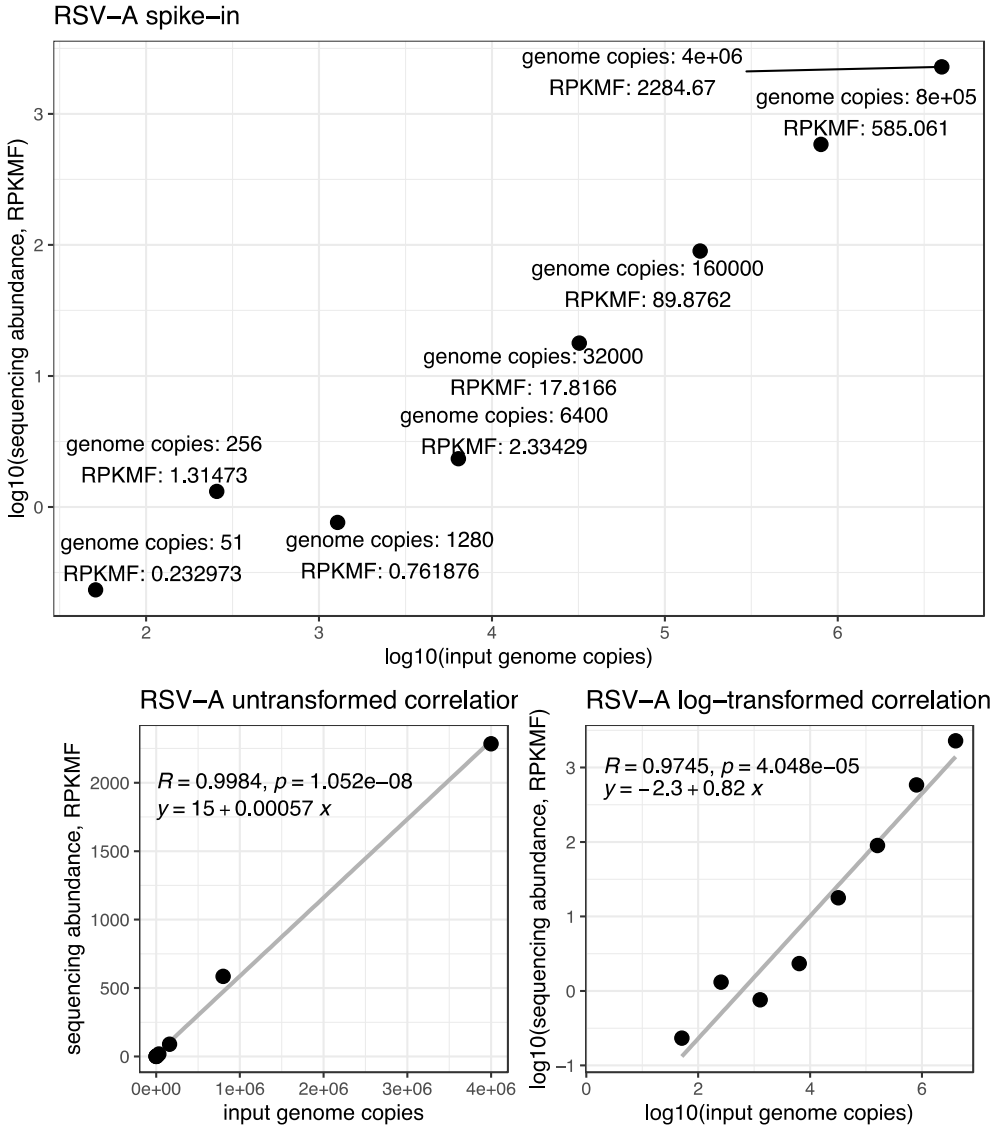
Fig. S1



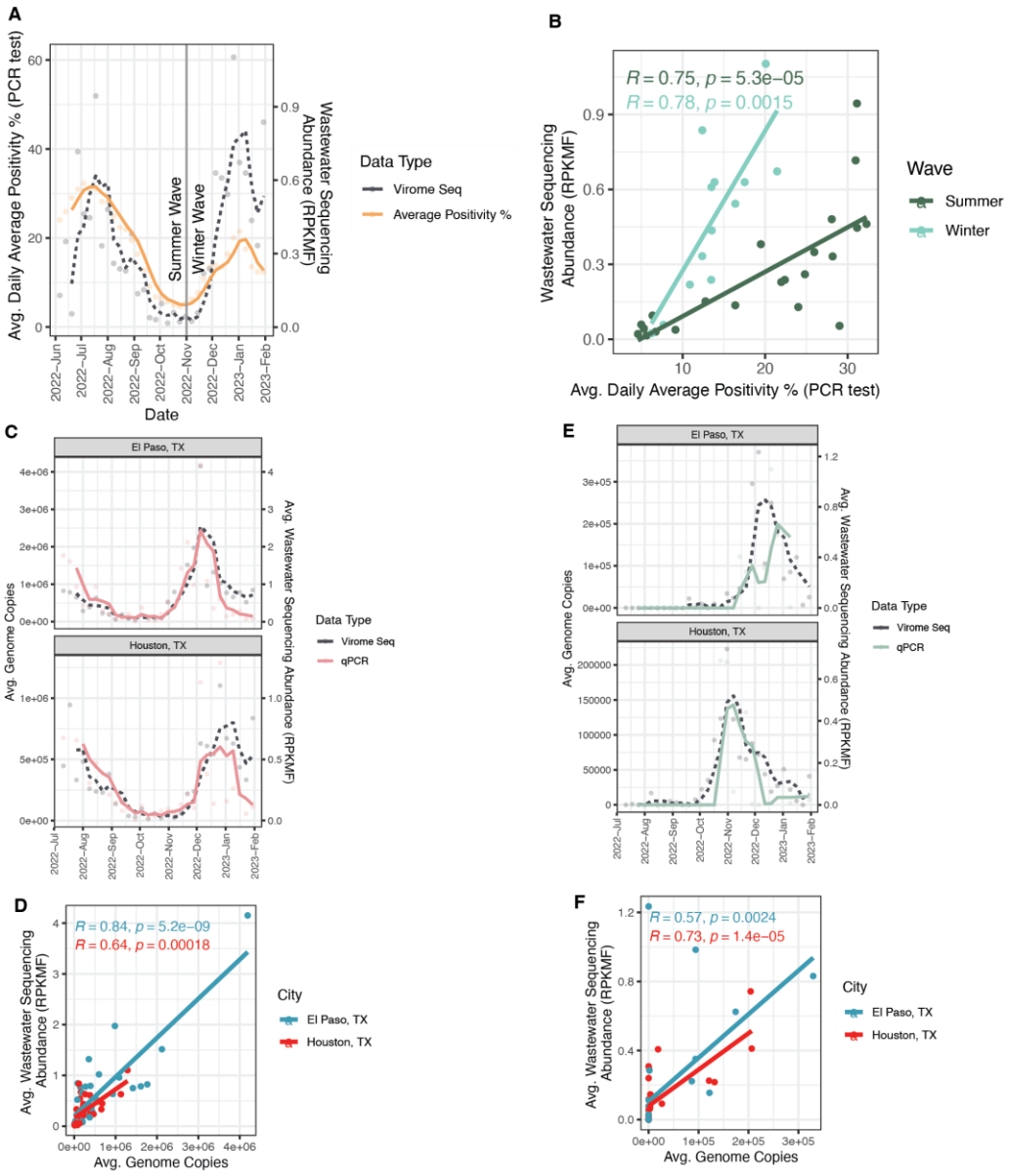
Schematic of EsViritu pipeline. EsViritu is suitable for detecting known and near-known mammalian virus genome sequences in metagenomic data. Relying on a de-replicated database of virus genomes, EsViritu aligns input reads to virus genomes with minimap2/coverM. For genomes that have read coverage of at least 1000 nucleotides or 50% of the sequence length, consensus sequences are extracted with SAMtools. Consensus sequences undergo all-vs-all pairwise comparison with BLASTn. Highly similar consensus sequences are de-replicated into the best (longest) exemplar. Reads are re-aligned to the de-replicated reference sequences with minimap2/coverM to calculate virus abundance metrics.

Fig. S2



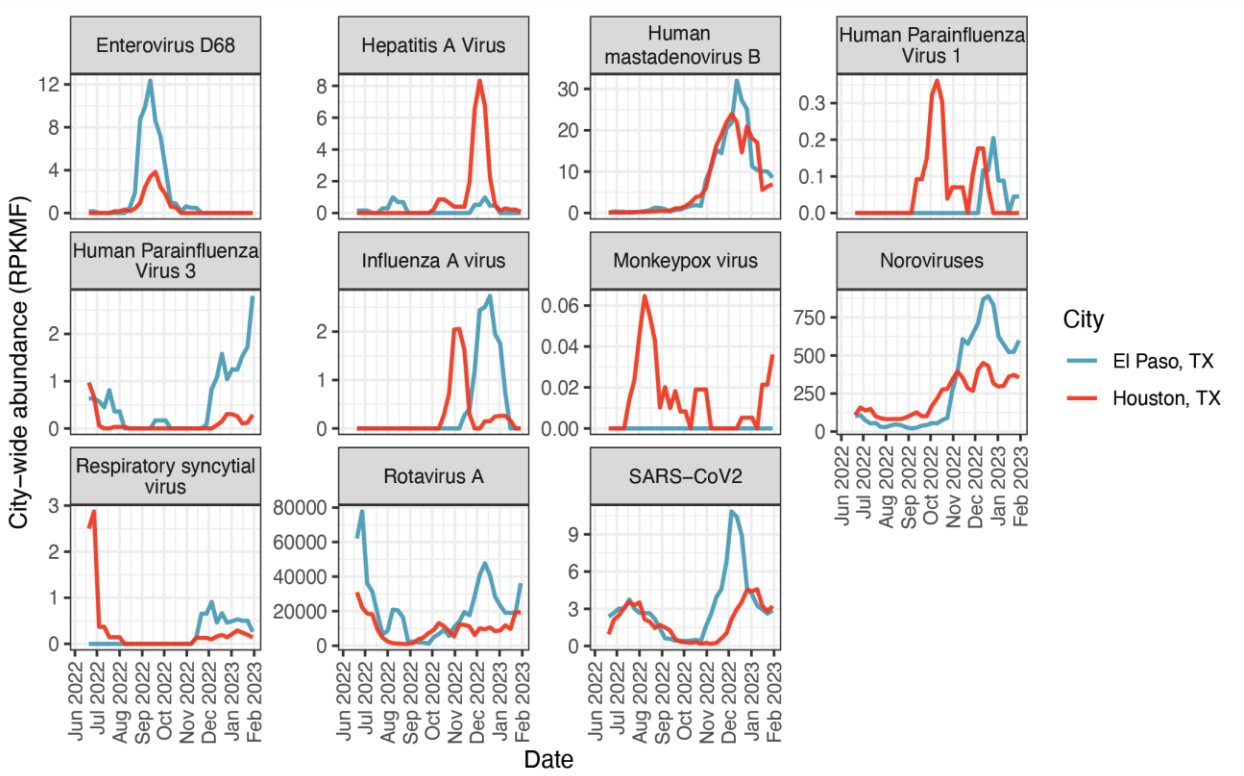
Dynamic range of pathogen detection in wastewater using RSV spike-in. All charts represent the results of an experiment spiking RSV into real wastewater samples. Wastewater samples were first processed with the hybrid-capture method described in this paper to screen for presence of RSV and only RSV-negative samples were used here. A 5:1 dilution series, starting with 4 million estimated genome copies down to 51 estimated genome copies, was prepared with wastewater samples, then processed with hybrid capture methods, sequenced, and quantified with EsViritu.

Fig. S3



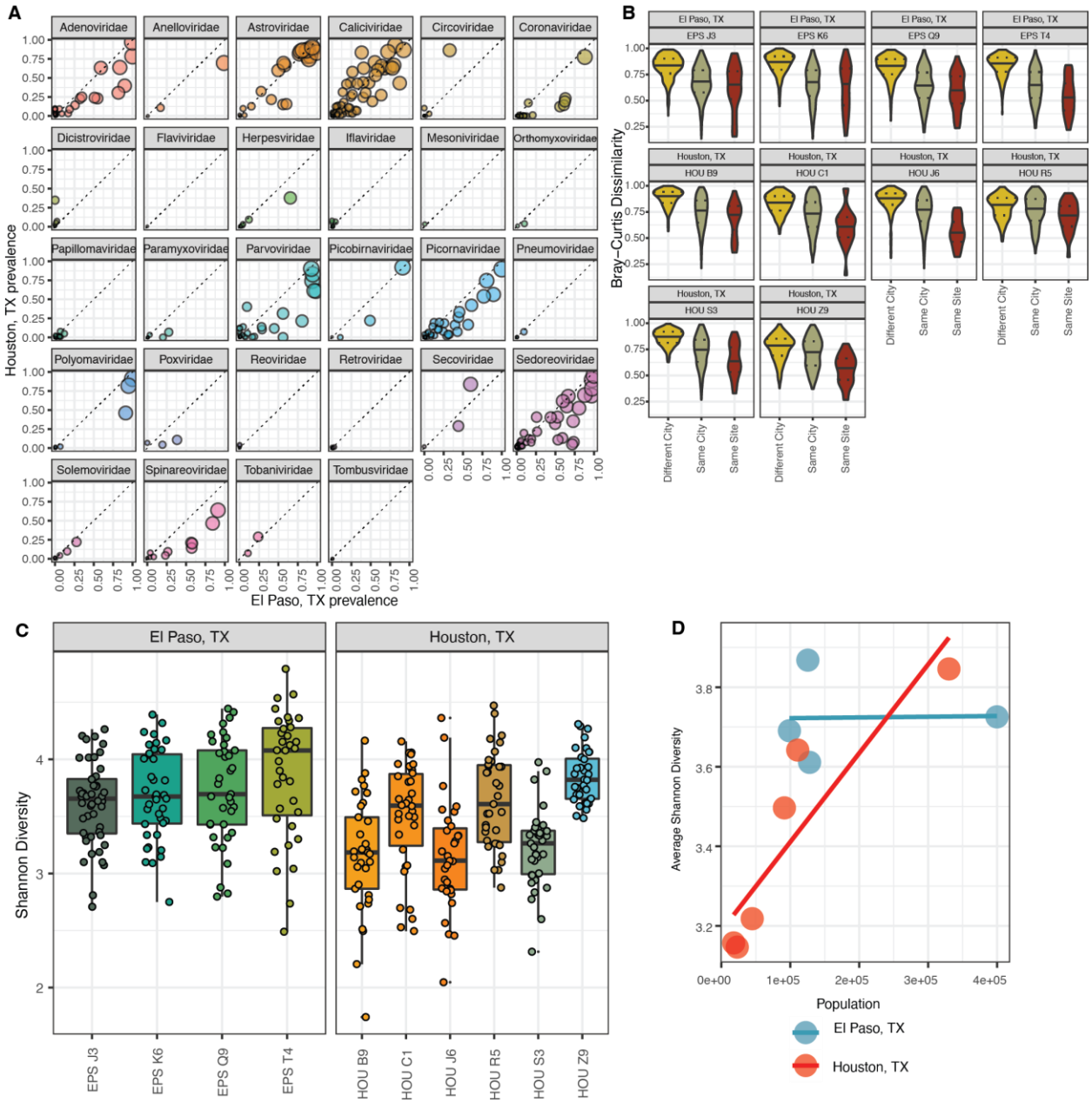
Comparison of virome sequencing to clinical data and qPCR of wastewater samples. (A) SARS-CoV-2 wastewater sequencing abundance compared to percent positivity of PCR tests in Houston, TX. **(B)** Scatter plot and correlation between wastewater sequencing abundance compared to percent positivity of PCR tests of SARS-CoV-2 in Houston, TX. **(C)** SARS-CoV-2 wastewater sequencing abundance compared qPCR values in Houston and El Paso, TX. **(D)** Scatter plot and correlation between wastewater sequencing abundance compared to qPCR values of SARS-CoV-2 in Houston and El Paso, TX. **(E)** Influenza A wastewater sequencing abundance compared qPCR values in Houston and El Paso, TX. **(F)** Scatter plot and correlation between wastewater sequencing abundance compared to qPCR values of Influenza A in Houston and El Paso, TX.

Fig. S4



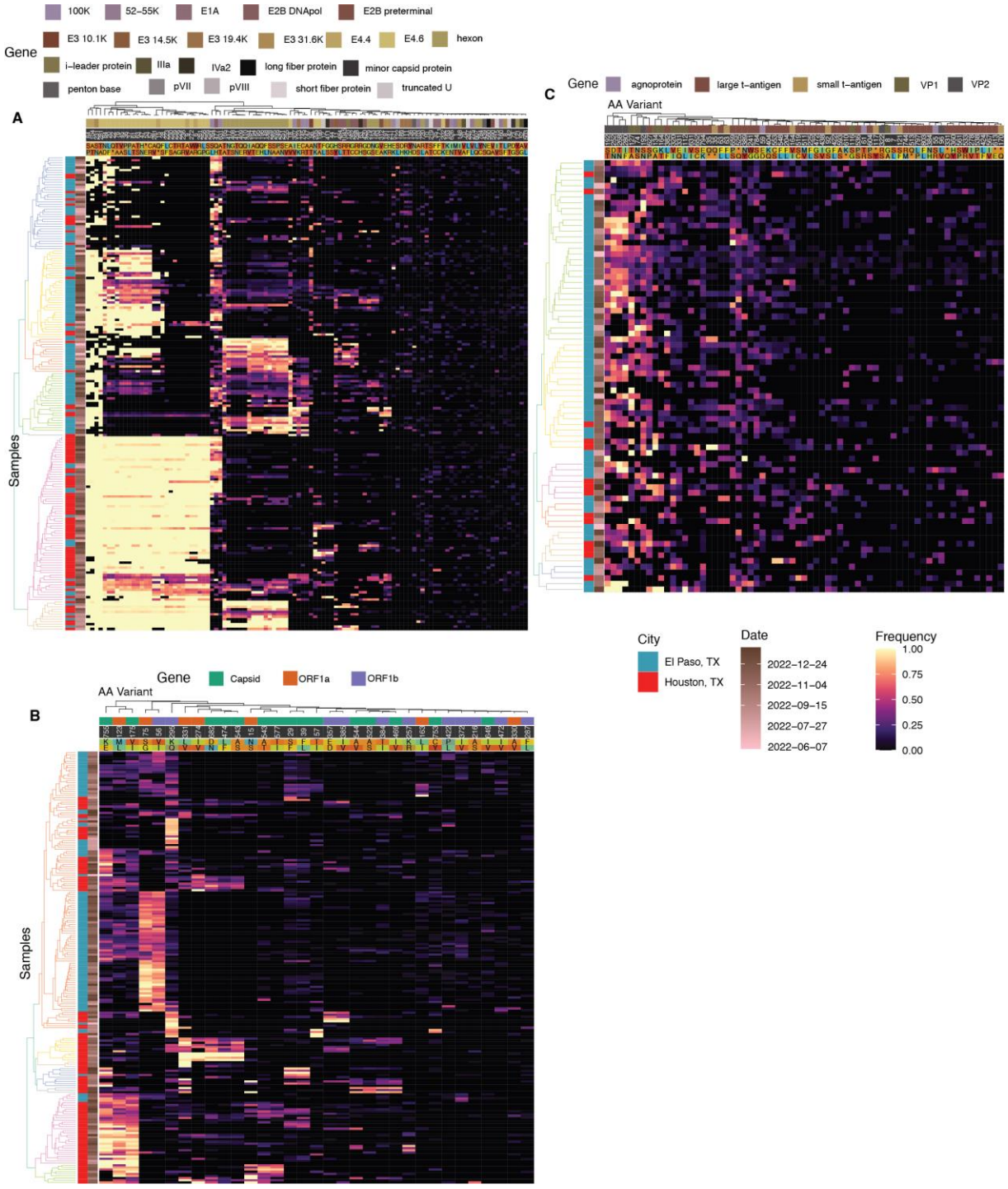
Virome sequencing Abundance of Major Pathogens. Moving average charts of eight virus pathogens in Houston and El Paso, TX.

Fig. S5



Additional wastewater virome community metrics. (A) All detected virus strains faceted by family with prevalence in Houston and El Paso. **(B)** Bray-Curtis dissimilarity between samples taken +/- seven days apart, comparing samples from the same site, different site but same city, and different city, faceted by site. **(C)** Shannon diversity by site. Boxplots are defined as: center line = median, lower and upper box-bounds = 25th and 75th data percentiles, and whiskers extend to the minimum and maximum values within 1.5 interquartile ranges. **(D)** Comparison of average Shannon diversity of wastewater treatment plants and population of catchment areas.

Fig. S6



Evaluation of non-synonymous variants in prevalent wastewater viruses (continued). (A) Dendrogram and heatmap describing frequency of common non-synonymous variants of astrovirus MLB1 by sample. **(B)** Like (A) but with Human Adenovirus 41. **(C)** Like (A) but with JC Polyomavirus.

Supplemental Table 1: Sequences and concentration of Influenza A, SARS-CoV-2 and RSV-A primers and probes standards used in RT-qPCR assay.

Primer	Sequence (5'-3')	Concentration
InfA Forward1	5'-CAAGACCAATCYTGTCACCTCTGAC-3'	3.3 µM
InfA Forward2	5'-CAAGACCAATYCTGTCACCTYTGAC-3'	3.3 µM
InfA Reverse1	5'-GCATTYTGGACAAAVCGTCTACG-3'	5 µM
InfA Reverse2	5'-GCATTTTGGATAAAGCGTCTACG-3'	1.67 µM
InfA probe	5'-/FAM/TGCAGTCCT/ZEN/CGCTCACTGGGCACG/3IABkFQ/-3'	1.67 µM
SC2-Forward	5'-CTGCAGATTTGGATGATTTCTCC-3'	6.67 µM
SC2-Reverse	5'-CCTTGTGTGGTCTGCATGAGTTTAG-3'	6.67 µM
SC2-Probe	5'-/TexRd-XN/ATTGCAACA/TAO/ATCCATGAGCAGTGCTGA CTC/3IAbRQSp/-3'	1.67 µM
RSV-A forward	5'-GATACACTCAACAAAGATCAACTTCTGTCA-3'	0.4 µM
RSV-A reverse	5'-AGGAGTGTCAATGCTGTCTCCTGTG-3'	0.4 µM
RSV-A probe	5'-/FAM/TCCAGCAAA/ZEN/TACACCATCCAACGGAG/3IABkFQ/-3'	0.2 µM
Influenza A oligonucleotide standard	5'-aatggctaaagacaagaccaatcctgtcacctctgactaa ggggattttaggatttgtgttcacgctcaccgtgcccagt gagcgaggactgcagcgtagacgctttgtccaaaatgccctcaatgggaa-3'	
RSVA oligonucleotide standard	5'-GAATGATACACTCAACAAAGATCAACTTCTGTCATCCAGCAAAT ACACCATCCAACGGAGCACAGGAGACAGCATTGACACTCCT-3'	