**Supplemental Information Titles and Legends**

***Supplemental Figure S1. PathSeq scores for all CNS-related viruses remained low, Related to Figure 2.*** (A) PathSeq scores for all CNS-related viruses detected in samples from the MSBB cohort are arranged by disease severity score. (B) All CNS virus detected in the ROSMAP cohort are arranged by individual, with no distinction between AD disease status.
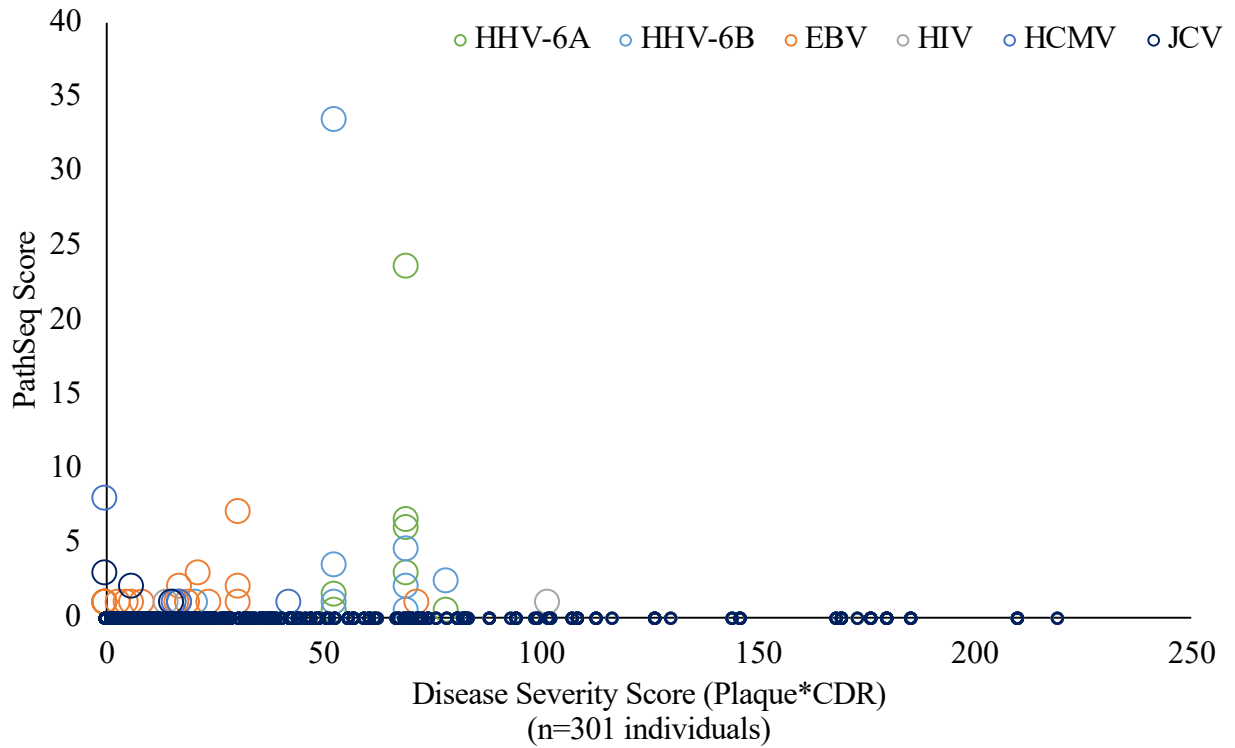
***Supplemental Table S1. Statistical analysis of viral detection between AD classifications, Related to Figure 2.*** The number of brains in which one or more brain regions were positive for the HHV-6 virus are displayed for both MSBB and ROSMAP RNAseq datasets. Chi-square ($\chi^2$) analysis of viral detection across AD classifications did not reveal any significant differences for any of the 6 CNS-related viruses detected.

***Supplemental Figure S2. Detection of ciHHV6 in ROSMAP and JHBRC cohorts, Related to Figure 3.*** (A) Individuals with a viral load around 1 copy/cell are consistent with chromosomal integration of HHV-6. In this triplex ddPCR assay, RPP30 and HHV-6B appear on the x-axis, while HHV-6A appears on the y-axis. (B) 5 total individuals were detected with viral loads between 0.9-1.2 copies HHV-6 DNA per cell, 3 individuals with ciHHV-6A and 2 with ciHHV-6B. These individuals were detected in both JHBRC and ROSMAP, as well as in both AD and non-AD controls.
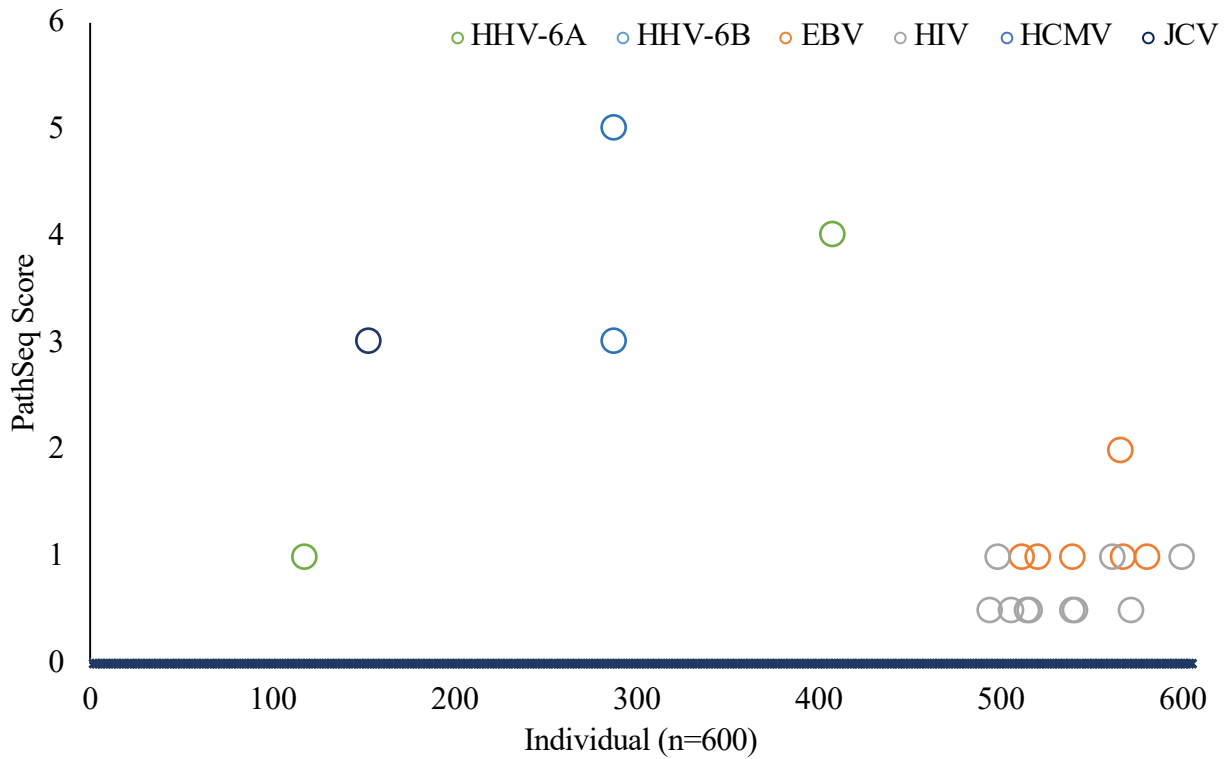
***Supplemental Table S2. Sex demographics of all subjects, Related to STAR Methods.*** Subjects across each of the four datasets used in the study were distributed between sexes, with approximately twice as many females as males in each cohort, and several subjects had unreported sex in the DNA ddPCR datasets.
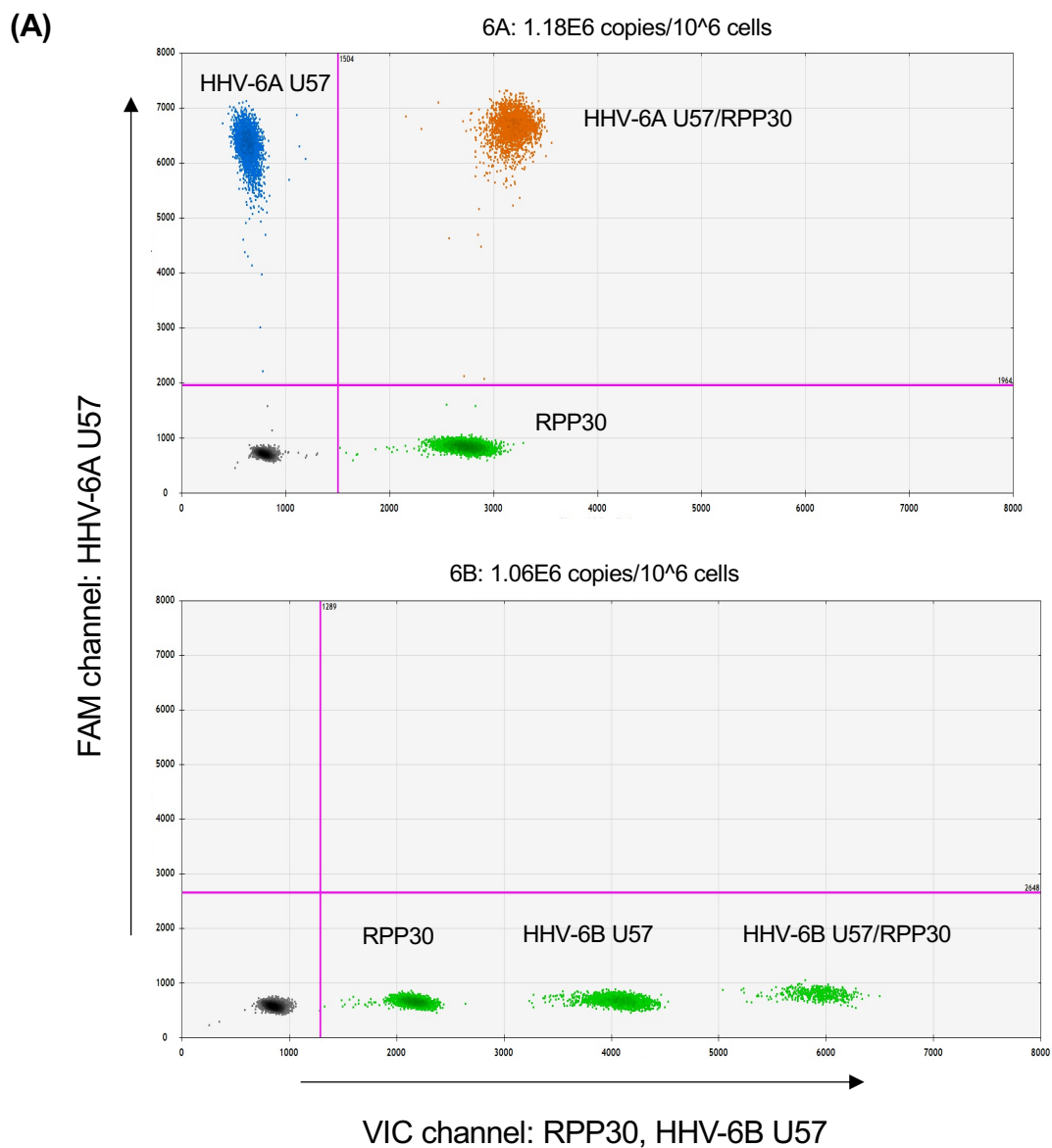
**Supplemental Figure 1**

**(A) MSBB**



**(B) ROSMAP**

**Supplemental Table 1**

| MSBB | Individuals (n) | HHV-6A | HHV-6B | CMV | EBV | HIV | JCV |
|---|---|---|---|---|---|---|---|
| **Definite AD** | 135 | 3 | 3 | 3 | 9 | 2 | 2 |
| **Probable AD** | 42 | 0 | 1 | 0 | 1 | 0 | 0 |
| **Possible AD** | 38 | 0 | 0 | 0 | 3 | 0 | 0 |
| **Non-AD controls** | 86 | 0 | 0 | 1 | 5 | 0 | 1 |
| $\chi^2$ **between groups (p=)** | | 0.293 | 0.416 | 0.590 | 0.721 | 0.480 | 0.765 |
| $\chi^2$ **All AD vs. controls (p=)** | | 0.271 | 0.203 | 0.874 | 0.939 | 0.369 | 0.854 |

| ROSMAP | Individuals (n) | HHV-6A | HHV-6B | CMV | EBV | HIV | JCV |
|---|---|---|---|---|---|---|---|
| **Definite AD** | 173 | 1 | 0 | 0 | 3 | 6 | 0 |
| **Probable AD** | 207 | 0 | 0 | 1 | 1 | 1 | 0 |
| **Possible AD** | 62 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Non-AD controls** | 158 | 1 | 0 | 1 | 2 | 3 | 1 |
| $\chi^2$ **between groups (p=)** | | 0.756 | - | 0.717 | 0.618 | 0.135 | 0.593 |
| $\chi^2$ **All AD vs. controls (p=)** | | 0.397 | - | 0.447 | 0.589 | 0.237 | 0.550 |

## Supplemental Figure 2

**(A)**



6A: 1.18E6 copies/10^6 cells

6B: 1.06E6 copies/10^6 cells

FAM channel: HHV-6A U57

VIC channel: RPP30, HHV-6B U57

**(B)**

|  | Religious Orders Study/Memory and Aging Project (ROSMAP) | | Johns Hopkins Brain Resource Center (JHBRC) | |
|---|---|---|---|---|
|  | Non-AD control | AD | Non-AD control | AD |
| **ciHHV-6A:** | 2/97 (2.1%) | 1/267 (0.4%) | 0/69 | 0/243 |
| **ciHHV-6B:** | 0/97 | 0/267 | 0/69 | 2/243 (0.8%) |

**Supplemental Table 2**

| | Mount Sinai Brain Bank (MSBB) | Religious Orders Study/Memory and Aging Project (ROSMAP) | | Johns Hopkins Brain Resource Center (JHBRC) |
|---|---|---|---|---|
| | RNAseq | RNAseq | DNA ddPCR | DNA ddPCR |
| | n=301 | n=600 | n=364 | n=344 |
| Female | 193 | 391 | 237 | 203 |
| Male | 108 | 211 | 119 | 139 |
| Unknown | - | - | 9 | 2 |