## SUPPLEMENTARY MATERIALS AND METHODS

### Swab collection

***Skin swabs from parapsoriasis patients:***

Swabs were collected under sterile conditions with a sterile swab (Copan Flocked Swab®, Copan Diagnostics Inc., Murrieta, CA, USA) as previously described (Salava et al. 2017). The swab was first dipped in a buffer solution (sterile 0.15 M NaCl with 0.1% Tween 20), then rubbed approximately 20 times back and forth over the target skin area of 5 x 5 cm on the PP patches or healthy skin on the contralateral sides of the same patient's body. Both the lesion and the healthy skin were sampled twice, yielding in total 52 swabs. Samples were deposited immediately in sterile 1.5-ml microcentrifuge tubes, frozen with liquid nitrogen, and stored at –80ºC until DNA extraction.

***Skin swabs from healthy individuals:***

Skin swab samples were collected from four different sites of each individual: forearm, inner elbow, behind the ear (retroauricular crease), and forehead, a total of 171 samples. Samples were taken with a sterile plastic swab stick (Eurotubo, Deltalab). The swab stick was wet inside a microcentrifuge tube containing 500 µl sterile buffer (0.9% NaCl with 0.1% Tween20) before sampling by rubbing back and forth on an area of approximately ≥ 2 cm x 2 cm of the target skin until skin was reddish. After sampling, the stick was placed back into the tube, and stored at -70°C from two to four months before nucleic acid extraction.

### DNA Extraction

DNA from parapsoriasis skin swabs was extracted with the FastDNA Spin Kit (MP Biomedicals, LLC, Santa Ana, CA, USA) according to the manufacturer's instructions. DNA from skin swabs of healthy individuals was extracted with QIAamp DNA mini kit (Qiagen, Germany). Carrier-RNA was included, as recommended by the manufacturer when the starting material is not rich in cells, which applies to the skin swabs. All DNA extractions were stored at −20°C until analyses. DNA from FFPE skin samples was extracted with QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) with some modifications to the manufacturer's instructions, as described previously (Väisänen et al. 2019). To avoid contamination, the FFPE blocks were cut with different blades, and the microtome was washed between the blocks with detergent, chlorite and ethanol.

The quality of DNA extraction and the number of cells in the PP skin samples were verified with the reference human gene *RNaseP* qPCR, as previously described (McNees et al. 2005). The healthy control skin swabs were initially studied with a *Luminex*-based *RNaseP* PCR, as described (Sadeghi et al. 2017). Of the 171 skin swab samples from the 51 healthy adults, 170 were positive for the human *RNaseP* gene, indicating that the DNA was intact. To confirm that the DNA yields were similar in swabs of PP and healthy subjects, random swab DNA extractions of all four skin sites from the healthy subjects were further repeated by *RNaseP* qPCR. The average number of cells/µl in the swabs of PP patients was 3.7 x 10^1, and from the healthy individuals, 6.48 x 10^1, respectively, verifying that the two swab analyses provided highly similar DNA recoveries.

### Quantitative PCRs and Sequencing

To prevent contamination, the master mix components, samples and plasmids were each handled in separate rooms. Laminar hoods were used for preparing and handling the samples and plasmids. Non-template controls were included in all PCR reactions. Ten-fold diluted plasmids ($10^0$-$10^6$), containing each viral target amplicon, served as standards and as positive controls.

***Protoparvoviruses:*** To detect and quantify protoparvovirus, CuV, bufavirus (BuV), and tusavirus (TuV) DNA, a multiplex real-time qPCR was performed with primers and hydrolysis probes located in the VP2 regions of CuV and TuV and the NS1 region of BuV, as previously described (Väisänen et al. 2019). Initial screenings were done in multiplex assays for all samples, and the positives (all of which were CuV) were repeated by singleplex qPCR. The PCR amplicons of 27 CuV DNA-positive samples (12 swabs and 15 biopsies) were further verified by cloning and sequencing. In addition, longer sequences (570 and 230 bp) could be obtained only from high viral load CuV DNA-positive

samples with respective primers CuV 583 fwd and CuV 230 fwd with CuV rev, as previously published (Väisänen et al. 2019). In our qPCR assays of FFPE extracts, we obtained ~580-bp long sequences from only two samples, ~230-bp sequences from seven samples, and 90-bp amplicons from twelve samples, which is in line with the well-known fragmentation of DNA in FFPE samples leading to lower sensitivity of PCRs with longer amplicons. Longer sequences from swab samples were not obtained due to insufficient amounts of sample. All sequences >200 bp long, are deposited in GenBank, accession no. OP751138 - OP751146.

***PCRs of other viruses:*** The amplification of parvovirus B19V, Merkel cell (MCPyV) and BK (BKPyV) polyomaviruses as well as nine human herpesviruses [herpes simplex viruses 1 and 2 (HSV-1 and -2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpesvirus 6A and 6B (HHV-6A, B), HHV-7, HHV-8)] were done with qPCRs, as previously described (Toppinen et al. 2015; Goh et al. 2009; Dumoulin and Hirsch 2011; Pyöriä et al. 2020).

***Sequence analysis:*** The sequencing results from skin biopsy samples can be seen in Figure S2. For individual P2, the two aligned 200-bp CuV-amplicon sequences, obtained from the parapsoriasis plaque (AS 2) and from the healthy skin (AS 15), were identical. For individual P5, a single nucleotide difference was observed between the CuV sequences of the healthy (AS18) and plaque (AS5) skin samples. There were no other notable differences in the CuV sequences, within the amplified region, between healthy and parapsoriasis en plaque skin biopsy samples of the same patients, but they differed between samples from different individuals, confirming that the sequences did not result from cross contamination, or from the plasmid control.

**RNAscope in-situ hybridization (ISH)**

RNAscope ISH (RISH) technology (Advanced Cell Diagnostics [ACD], Newark, CA) was used for viral RNA or DNA detection on 5-μm FFPE tissue sections cut on glass slides (SuperFrost Plus) using Probe-V-CuV-gp3-gp4-gp5 [NC_039050.1 (2023 – 3562 bp] targeting the sense DNA strand in the *VP* region. Note: antisense probes may detect both viral DNA and mRNA.
To test the specificity of CuV *VP* RISH probes, besides the CuV PCR-negative skin control (Fig 1C), also H5 insect cells, uninfected and infected with a CuV *VP2* gene- (GenBank no. MH127919) containing baculovirus (Väisänen et al. 2018), were cytospinned on glass slides for consecutive RISH probe testing (Figure S3).

**Statistical Analysis**

Statistics were calculated with Fisher exact test. The limit for statistically significant differences was $p \leq 0.05$.

## SUPPLEMENTARY TABLES AND FIGURES

**Table S1**: Age group-related prevalence of CuV DNA in skin swabs from healthy and plaque-type parapsoriasis (PP) individuals.

| Age range | **CuV+/total PP individuals** | Prevalence in PP (%) | **CuV+/total healthy individuals** | Prevalence in healthy controls (%) | **Significance (P value)** |
|---|---|---|---|---|---|
| **18-86** | 6/13 | 46.2 | 1/51 | 1.96 | 0.0001 |
| **18-36** | 0/0 | 0 | 0/23 | | |
| **37-65** | 3/6 | 50 | 1/28 | 3.57 | 0.0124 |
| **>65** | 5/7 | 71.4 | 0 | | |

PP, parapsoriasis. The CuV-DNA prevalence among skin swabs from parapsoriasis patients (46.2%, 6/13) was significantly higher (p =0.0001, 95% CI: 0.174, 0.706) than from the healthy controls (1.96%, 1/51) with a risk difference (RD) of 0.44. The difference remained significant (p =0.0124, 95% CI: 0.06, 0.87) when comparing only the 37-65-year-olds common to both groups, even though the numbers were small; 3/6 (50%) vs. 1/28 (3.5%). P values were obtained by Fisher exact test, with p<0.5 being significant.

**Table S2:** Parapsoriasis en plaques patients with CuV DNA in skin swab or biopsy samples.

| Patient | Age/ Gender | Diagnosis and year of diagnosis | Skin sample type | Site of sampling | Sample ID | CuV copies/ one million cells |
|---|---|---|---|---|---|---|
| P2 | 73/M | SPP 2002 | Swab | PP plaque | PP 5 and 6 | 2.69E+06; 7.16E+05 |
| | | | | Healthy | PP 7 and 8 | 5.04E-01*; Neg |
| | | | Biopsy | PP plaque | AS 2 | 9.68E+06 |
| | | | | Healthy | AS 15 | 2.39E+05 |
| P3 | 86/M | LPP 2011 | Swab | PP plaque | PP 9 and 10 | Neg, Neg |
| | | | | Healthy | PP 11 and 12 | Neg, Neg |
| | | | Biopsy | PP plaque | AS 3 | 5.66E+04 |
| | | | | Healthy | AS 16 | 2.49E+04 |
| P5 | 47/M | SPP 2003 | Swab | PP plaque | PP 17 and 18 | 8.60E+04; 2.91E+04 |
| | | | | Healthy | PP 19 and 20 | 2.96E+06; 5.22E+05 |
| | | | Biopsy | PP plaque | AS 5 | 5.68E+06 |
| | | | | Healthy | AS 18 | 5.66E+05 |
| P6 | 69/F | LPP 2010 | Swab | PP plaque | PP 21 and 22 | Neg; Neg |
| | | | | Healthy | PP 23 and 24 | Neg; Neg |
| | | | Biopsy | PP plaque | AS 6 | Neg |
| | | | | Healthy | AS 19 | 1.14E+06 |
| P7 | 66/F | SPP 2008/ Pre-MF 2023 | Swab | PP plaque | PP25 and 26 | Neg; 4.05E+06 |
| | | | | Healthy | PP 27 and 28 | 2.86E+07; 5.00E+00* |
| | | | Biopsy | PP plaque | AS 7 | 1.47E+07 |
| | | | | Healthy | AS 20 | 2.71E+06 |
| P9 | 71/M | SPP 2010 | Swab | PP plaque | PP 33 and 34 | 1.96E+06; 1.51E+06 |
| | | | | Healthy | PP 35 and 36 | 7.71E+04; Neg |
| | | | Biopsy | PP plaque | AS 9 | 2.14E+07 |
| | | | | Healthy | AS 22 | 4.00E+05 |
| P11 | 57/M | LPP 2001 | Swab | PP plaque | PP 41 and 42 | 5.65E+05; 1.06E+06 |
| | | | | Healthy | PP 43 and 44 | Neg; 3.22E+05 |
| | | | Biopsy | PP plaque | AS 11 | 2.67E+07 |
| | | | | Healthy | AS 24 | 3.93E+04 |
| P12 | 59/F | LPP 2002/ MF 2017 | Swab | PP plaque | PP 45 and 46 | 1.37E+07; 2.06E+06 |
| | | | | Healthy | PP 47 and 48 | 1.12E+07; 1.58E+07 |
| | | | Biopsy | PP plaque | AS 12 | 5.88E+07 |
| | | | | Healthy | AS 25 | 1.64E+06 |

* CuV copies/ul of extraction. PP, parapsoriasis. All samples were RNaseP-PCR positive. All CuV-positive patients, except P6, exhibited higher viral loads in biopsies from PP than from healthy skin, but this did not reach significance.

**Figure S1.** Alignment of 91-nt CuV sequences from skin swabs in the current study. Dot (.) indicates identical nucleotides. 48R1 (forearm), 48R3 (forehead) and 48R4 (behind ear) are samples from the one healthy individual who had CuV DNA-positive skin swabs. Numbers after the sample number indicate the bacterial colony which was used for plasmid isolation and consequent sequencing. From the same patient swabs, sequences with one or two mismatches were obtained from different colonies. All sequences had differences from that of the control plasmid.

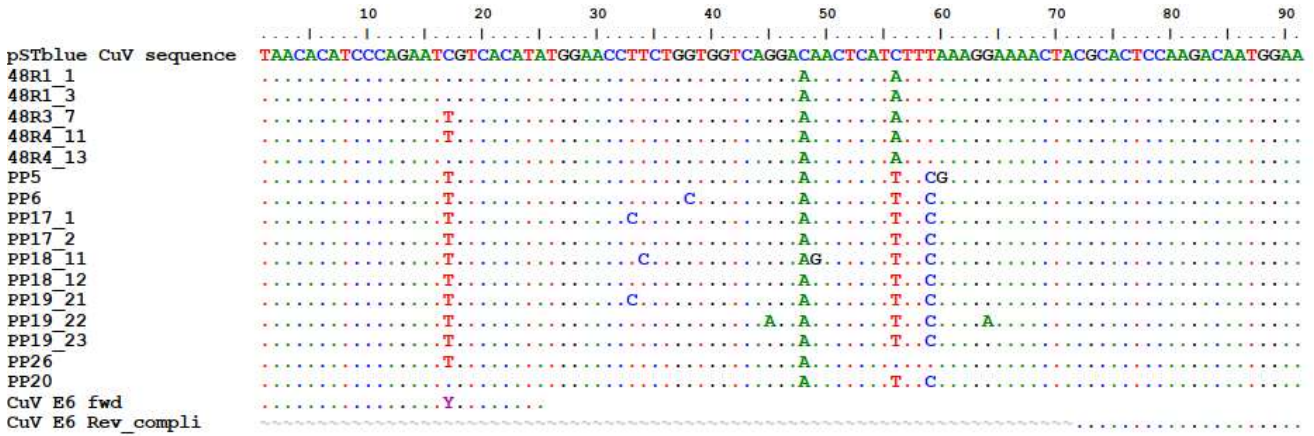


**Figure S2.** Alignment of the 200-nt CuV DNA sequences from skin biopsy samples from eight parapsoriasis patients (from healthy and parapsoriasis skin regions of two patients) to the reference sequence NC_039050.1 (4117 – 4316 nt). Dots (.) indicate identical nucleotides.

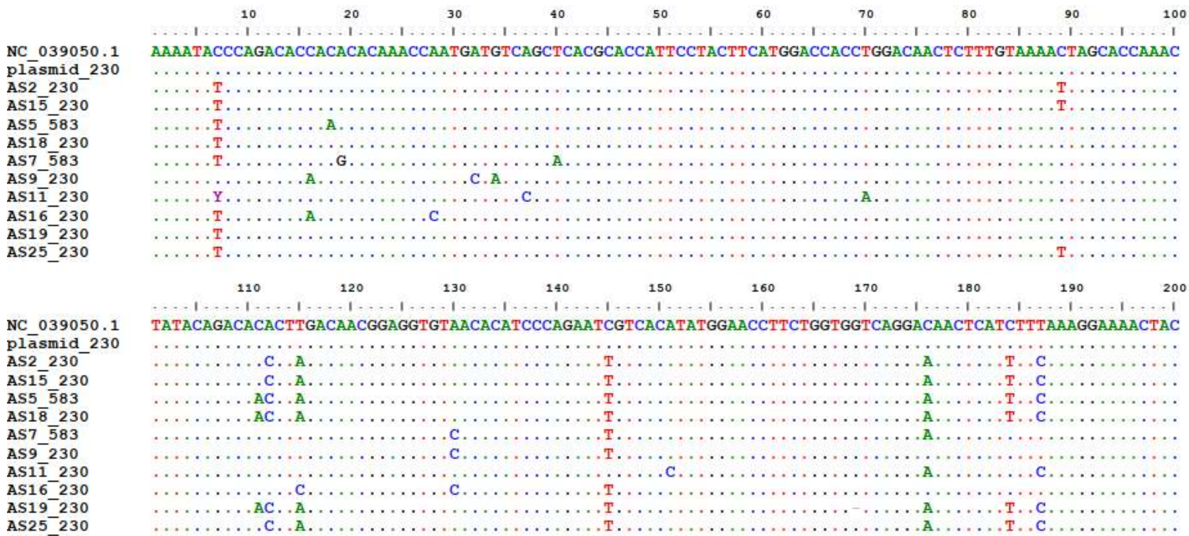
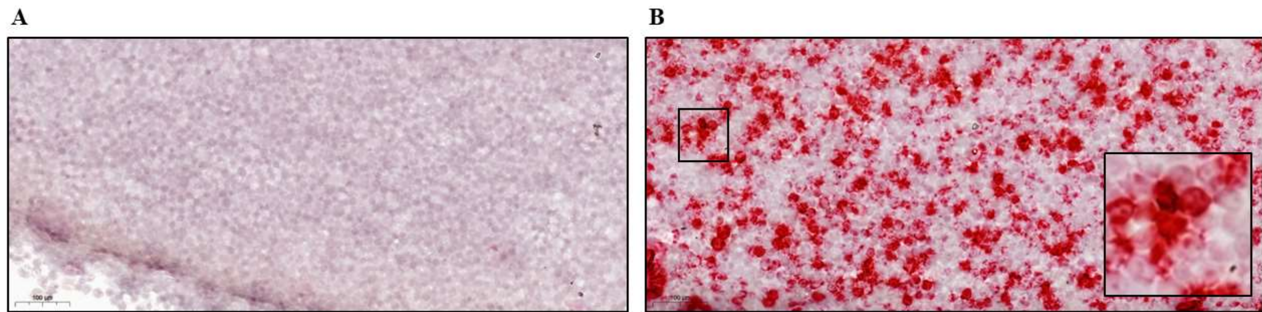


**Figure S3.** RNAscope ISH RED assay with the CuV *VP* probe on H5 insect cells (A) non infected and (B) infected with baculovirus expressing CuV *VP2*. Of note, the anti-sense *VP* probe will detect both viral mRNA and sense-strand DNA. Zoomed-in areas are shown in the bottom-right corners. Scale bars, 100 µm.




**Supplementary References**

Dumoulin A, Hirsch HH. Reevaluating and optimizing polyomavirus BK and JC real-time PCR assays to detect rare sequence polymorphisms. J. Clin. Microbiol. 2011;49(4):1382–8

Goh S, Lindau C, Tiveljung-Lindell A, Allander T. Merkel cell polyomavirus in respiratory tract secretions. Emerg Infect Dis 2009;15:489–91

McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS. Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J. Clin. Virol. 2005;34(1):52–62

Pyöriä L, Jokinen M, Toppinen M, Salminen H, Vuorinen T, Hukkanen V, et al. HERQ-9 Is a New Multiplex PCR for Differentiation and Quantification of All Nine Human Herpesviruses. mSphere 2020;5:e00265-20.

Sadeghi M, Wang Y, Ramqvist T, Aaltonen L-M, Pyöriä L, Toppinen M, et al. Multiplex detection in tonsillar tissue of all known human polyomaviruses. BMC Infect Dis 2017;17:409

Salava A, Pereira P, Aho V, Väkevä L, Paulin L, Auvinen P, et al. Skin microbiome in small- and large-plaque parapsoriasis. Acta Derm Venereol 2017;97:685–91

Toppinen M, Norja P, Aaltonen LM, Wessberg S, Hedman L, Söderlund-Venermo M, et al. A new quantitative PCR for human parvovirus B19 genotypes. J Virol Methods 2015;218:40–5

Väisänen E, Mohanraj U, Kinnunen PM, Jokelainen P, Al-Hello H, Barakat AM. Global distribution of human protoparvoviruses. *Emerg Infect Dis* 2018; *24*(7):1292.

Väisänen E, Fu Y, Koskenmies S, Fyhrquist N, Wang Y, Keinonen A, et al. Cutavirus DNA in malignant and nonmalignant skin of cutaneous t-cell lymphoma and organ transplant patients but not of healthy adults. Clin Infect Dis 2019;68:1904–10