Supplementary Figure 1. Viral Proteins and vRNA expression level in whole cells.

(a) Proteins from lysates of PV- and mock-infected cells at 3, 4, 5, 6, and 7 hours post infection (hpi) were probed by western blot. The blot was first probed for GAPDH (top panel) to show similar loading in all lanes. The blot was then stripped, and re-probed using polyclonal anti-3D antibodies. All species containing the 3D protein are expected to be labeled by the anti-3D antibody (e.g. Garmarnik & Andino (1998). Genes and Development 12:2293-2304, and Teterina et al., (2001). J. Virology 75:3841-3850); these additional species are shown in parentheses.

(b) Proteins from lysates of mock-infected (3 and 7 hpi) and PV-infected cells (3, 4, 5, 6, and 7 hpi) were probed for PV 3A; it is expected that 3AB is also labeled by this antibody, as indicated by parentheses.

(c) Proteins from lysates of PV-infected (Inf) and mock-infected (Mock) cells at 7 hpi were probed for PV 2C.

3AB: purified PV 3AB protein
BI: blank lanes
MW: molecular weight markers.
Supplementary Figure 2. Viral proteins are present in vesicles secreted by virus-infected cells.

Full western blots from Figure 1d, showing the presence of viral proteins in microvesicles secreted by PV-infected HeLa cells at 8 hours post infection (hpi), but not in vesicles isolated from mock-infected cells.

Vesicles were run on tricine gels, and probed with (a) anti-VP capsid protein antibodies; the membrane on the left is shown for its molecular weight markers (MW) and poliovirus stock.
solution, (b) anti-C antibodies; Mock 1 (vesicles from mock-infected cells) and Infect 1 (vesicles from PV-infected cells) are from one experiment, and Mock 2 and Infect 2 are from an independent experiment, (c) anti-3D antibodies; Mock prep 1 (vesicles from mock-infected cells) and Infected prep 1 (vesicles from PV-infected cells) are from one experiment, and Mock prep 2 and Infected prep 2 are from an independent experiment, (d) anti-3A antibodies; lanes are samples of vesicles secreted by mock- and PV-infected cells at 7 hpi. All species containing the protein probed for are expected to be labeled by that antibody (see, e.g., Garmarnik & Andino (1998). Genes and Development 12:2293-2304, and Teterina et al., (2001). J. Virology 75:3841-3850); these additional species are shown in parentheses. MW: molecular weight markers.
Supplementary Figure 3 b-k (panel a is blank)

Supplementary Figure 3. Viral Proteins and vRNA expression level in whole cells. (b-i) Confirmation and determination of specificity of the positive- and negative-strand RNA assays show no amplification in PV-infected cells when reverse transcriptase was absent, probed
using positive- (b) and negative- (c) sense RNA strands. No amplification was observed in water, probed using positive- (d) and negative- (e) sense RNA. Primers were able to specifically recognize positive- (f) and negative- (g) sense viral RNA from the total RNA extracted from PV-infected cells. The single peak in the melt curve of cDNA products of positive- (h) and negative- (i) sense viral RNA probes from PV-infected cells indicates the presence of one target amplicon. The amplification of positive-sense viral RNA was absent in mock-infected cells (j). The total RNA of PV- (IW) and mock-infected (MW) cells at 8 hpi was collected, and the levels of (+) vRNA and (-) vRNA were determined using RT-qPCR (k). The y-axis indicates fluorescent intensity signal in arbitrary units. Panels b, c are expressed in log mode. Panels d-j are expressed in linear mode. RT-qPCR relative quantification was calculated as $\Delta C_t$ where $\Delta C_t = (C_t$ of endogenous control gene (GAPDH)) – (Ct of gene of interest (vRNA)), using GAPDH of whole cells for normalization.

Supplementary Figure 4. PV infectivity before and after the treatment of freeze-thaw, detergent, RNAse.

The titer of poliovirus stock solutions was determined by plaque assay before (black) and after (white) freeze-thaw, detergent, and RNAse treatment. The y-axis indicates the viral titer (plaque-forming units) while the x-axis indicates three independent experiments.
Supplementary Figure 5. Virions were rarely seen dispersed within the bundled actin filaments carried by infectious microvesicles.

Successive 1 nanometer thick slices through a reconstructed cryo-electron tomogram (a-d). Traced models of (a’-d’) depict the disappearance and appearance of individual virions (red hexagons) among actin filaments (black arrows). The scale bar is 100 nm.

Supplemental Movie. Cryotomogram of a microvesicle from poliovirus-infected cells

Movie of an electron cryotomogram of a Class III microvesicle isolated from poliovirus-infected cells. Labeled, and later shown in isosurface-rendered models in the tomogram, are virions, vesicle membranes, mat-like structures, and the inner vesicular structure. Additional labels indicate representative annexin-V beads that were used to isolate the sample, and fiducial gold beads that were added to aid alignment of tilt series data. Scale bar, 100 nm.
## Supplemental Table S1.

Proteins Identified in Microvesicles Isolated from Cells that were:

### PV-infected

| Accession # | Protein Abbreviation | P | Aggregated Area from one run of ImVs | Description | Aggregated Area from one run of ImVs |
|-------------|----------------------|---|--------------------------------------|-------------|--------------------------------------|
| P60709      | ACTB_HUMAN           | 378.78 | 1.32E-38 | 97500000 | Actin cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 |
| P63261      | ACTG_HUMAN           | 378.78 | 1.32E-38 | 97500000 | Actin cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1 |
| P80723      | BASP1_HUMAN          | 370.94 | 8.05E-38 | 28800000 | Brain acid soluble protein 1 OS=Homo sapiens GN=BASP1 PE=1 SV=2 |
| P35908      | K22E_HUMAN           | 330.12 | 9.73E-34 | 51700000 | Keratin type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 |
| P04264      | K2C1_HUMAN           | 307.63 | 1.73E-31 | 48800000 | Keratin type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 |
| P50995      | ANXK1_HUMAN          | 269.92 | 1.02E-27 | 14800000 | Annexin A11 OS=Homo sapiens GN=ANXK11 PE=1 SV=1 |
| P13645      | K1C10_HUMAN          | 266.4  | 2.29E-27 | 41600000 | Keratin type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 |
| P35613      | BASI_HUMAN           | 253.39 | 4.58E-26 | 24400000 | Basigin OS=Homo sapiens GN=BSG PE=1 SV=2 |
| P02533      | K1C14_HUMAN          | 237.36 | 1.84E-24 | 30700000 | Keratin type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4 |
| P04406      | G3P_HUMAN            | 227.96 | 1.60E-23 | 92700000 | Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 |
| P11142      | HSP7C_HUMAN          | 225.22 | 3.01E-23 | 14600000 | Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 |
| P04083      | ANXA1_HUMAN          | 218.34 | 1.47E-22 | 31700000 | Annexin A1 OS=Homo sapiens GN=ANXA1 PE=1 SV=2 |
| P08195      | 4F2_HUMAN            | 214.05 | 3.94E-22 | 11100000 | 4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLCA2 PE=1 SV=2 |
| P07540      | PDCD6_HUMAN          | 213.31 | 4.67E-22 | 21300000 | Programmed cell death protein 6 OS=Homo sapiens GN=PDCD6 PE=1 SV=1 |
| P35527      | K1C9_HUMAN           | 206.24 | 2.38E-21 | 17800000 | Keratin type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 |
| P23525      | C0F3_HUMAN           | 204.53 | 3.52E-21 | 19300000 | Cofilin 1 OS=Homo sapiens GN=CFL1 PE=1 SV=3 |
| P05023      | AT1A1_HUMAN          | 204.08 | 3.91E-21 | 25300000 | Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1 PE=1 SV=1 |
| P08174      | DAF_HUMAN            | 190.48 | 8.95E-20 | 21600000 | Complement decay-accelerating factor OS=Homo sapiens GN=CD55 PE=1 SV=4 |
| P13987      | CD59_HUMAN           | 190.45 | 9.02E-20 | 61500000 | CD59 glycoprotein OS=Homo sapiens GN=CD59 PE=1 SV=1 |
| P09525      | ANXA4_HUMAN          | 181.51 | 7.08E-19 | 19800000 | Annexin A4 OS=Homo sapiens GN=ANXA4 PE=1 SV=4 |
| P26038      | MOE5_HUMAN           | 178.96 | 1.27E-18 | 23500000 | Moesin OS=Homo sapiens GN=MSN PE=1 SV=3 |
| P62937      | PPIA_HUMAN           | 173.59 | 4.38E-18 | 40700000 | Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 |
| P60174      | TP5_HUMAN            | 164.69 | 3.40E-17 | 27000000 | Triosephosphate isomerase OS=Homo sapiens GN=TP1 PE=1 SV=3 |
| P30626      | SORC1_HUMAN          | 157.35 | 1.84E-16 | 42900000 | Sorcin OS=Homo sapiens GN=SRI PE=1 SV=1 |
| P07195      | LDHB_HUMAN           | 156.21 | 2.39E-16 | 9910000  | l-Lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=1 |
| P61586      | RHDA_HUMAN           | 152.91 | 5.12E-16 | 6180000  | Transforming protein Rhod OS=Homo sapiens GN=RHDA PE=1 SV=1 |
| Q09666      | AHNK_HUMAN           | 150.48 | 8.95E-16 | 7160000  | Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens GN=AHNAK PE=1 SV=2 |
| P08238      | H590B_HUMAN          | 150.15 | 9.66E-16 | 15800000 | Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 |
| P63104      | 1433Z_HUMAN          | 147.65 | 1.72E-15 | 12000000 | 14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1 |
| P62087      | RL40_HUMAN           | 144.04 | 3.94E-15 | 54000000 | Ubiquitin-40S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2 |
| P62979      | RS27A_HUMAN          | 144.04 | 3.94E-15 | 54000000 | Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2 |
| P0C548      | UBC_HUMAN            | 144.04 | 3.94E-15 | 54000000 | Polyubiquitin-C OS=Homo sapiens GN=UBC PE=1 SV=3 |
| P06733      | ENOA_HUMAN           | 142.93 | 5.09E-15 | 23900000 | Alpha-epsilon OS=Homo sapiens GN=ENO1 PE=1 SV=2 |
| P08758      | ANXK3_HUMAN          | 142.1  | 6.17E-15 | 24600000 | Annexin A5 OS=Homo sapiens GN=ANXK3 PE=1 SV=2 |
| P16070      | CD44_HUMAN           | 141.89 | 6.47E-15 | 5710000  | CD44 antigen OS=Homo sapiens GN=CD44 PE=1 SV=3 |
| Q05274      | LYPD3_HUMAN          | 141.14 | 7.69E-15 | 4970000  | Ly6/PLAUR domain-containing protein 3 OS=Homo sapiens GN=LYPD3 PE=1 SV=2 |
| P02786      | TF2L_HUMAN           | 140.98 | 7.98E-15 | 7890000  | Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2 |
| P31949      | S10A8_HUMAN          | 140.88 | 8.17E-15 | 52900000 | Protein S100-A11 OS=Homo sapiens GN=S100A11 PE=1 SV=2 |
| P04075      | ALDOA_HUMAN          | 140.39 | 9.14E-15 | 10700000 | Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2 |
| P14618      | KPYM_HUMAN           | 138.78 | 1.32E-14 | 17500000 | Pyruvate kinase PM5 OS=Homo sapiens GN=PKM PE=1 SV=4 |

### Mock-infected
Supplemental Table S1 (continued).
Proteins Identified in Microvesicles Isolated from Cells that were:

| Protein ID | Protein Name | Accession Number | Monoisotopic Mass | Charge State | Protein Name | Accession Number | Monoisotopic Mass | Charge State |
|------------|--------------|------------------|-------------------|--------------|--------------|------------------|-------------------|--------------|
| QBW5M4     | PDC6I_HUMAN  | OS=Homo sapiens  | 138.08            | 1.56E-14     | 69600000     | PDC6I_HUMAN      | OS=Homo sapiens  | 138.08       |
| P20073     | ANS7_HUMAN   | OS=Homo sapiens  | 135.42            | 2.87E-14     | 13500000     | ANS7_HUMAN       | OS=Homo sapiens  | 135.42       |
| P39004     | L1CAM_HUMAN  | OS=Homo sapiens  | 134.29            | 3.72E-14     | 50200000     | L1CAM_HUMAN      | OS=Homo sapiens  | 134.29       |
| P07437     | TUBB5I_HUMAN | OS=Homo sapiens  | 129.88            | 1.03E-13     | 46700000     | TUBB5I_HUMAN     | OS=Homo sapiens  | 129.88       |
| P15311     | EZR_HUMAN    | OS=Homo sapiens  | 129.76            | 1.06E-13     | 40900000     | EZR_HUMAN        | OS=Homo sapiens  | 129.76       |
| O75131     | CPNE3_HUMAN  | OS=Homo sapiens  | 123.29            | 4.69E-13     | 10200000     | CPNE3_HUMAN      | OS=Homo sapiens  | 123.29       |
| P00338     | LDHA_HUMAN   | OS=Homo sapiens  | 121.52            | 7.05E-13     | 75800000     | LDHA_HUMAN       | OS=Homo sapiens  | 121.52       |
| P05556     | ITB1_HUMAN   | OS=Homo sapiens  | 120.67            | 8.57E-13     | 95400000     | ITB1_HUMAN       | OS=Homo sapiens  | 120.67       |
| P0DMV8     | HS71A_HUMAN  | OS=Homo sapiens  | 118.29            | 1.48E-12     | 35300000     | HS71A_HUMAN      | OS=Homo sapiens  | 118.29       |
| P0DMV9     | HS71B_HUMAN  | OS=Homo sapiens  | 118.29            | 1.48E-12     | 35300000     | HS71B_HUMAN      | OS=Homo sapiens  | 118.29       |
| Q9UBV8     | PFEF1_HUMAN  | OS=Homo sapiens  | 118.08            | 1.56E-12     | 25400000     | PFEF1_HUMAN      | OS=Homo sapiens  | 118.08       |
| P10809     | CHG0_HUMAN   | OS=Homo sapiens  | 114.89            | 3.24E-12     | 30200000     | CHG0_HUMAN       | OS=Homo sapiens  | 114.89       |
| Q16658     | FSCN1_HUMAN  | OS=Homo sapiens  | 112.11            | 6.17E-12     | 64600000     | FSCN1_HUMAN      | OS=Homo sapiens  | 112.11       |
| P08754     | GNAI3_HUMAN  | OS=Homo sapiens  | 111.38            | 7.28E-12     | 40600000     | GNAI3_HUMAN      | OS=Homo sapiens  | 111.38       |
| O0X099     | CLIC1_HUMAN  | OS=Homo sapiens  | 96.21             | 2.39E-10     | 29900000     | CLIC1_HUMAN      | OS=Homo sapiens  | 96.21        |
| Q9H444     | CHM4B_HUMAN  | OS=Homo sapiens  | 95.61             | 2.75E-10     | 50800000     | CHM4B_HUMAN      | OS=Homo sapiens  | 95.61        |
| P08133     | ANXA6_HUMAN  | OS=Homo sapiens  | 91.15             | 7.67E-10     | 13800000     | ANXA6_HUMAN      | OS=Homo sapiens  | 91.15        |
| Q06830     | PRDXI_HUMAN  | OS=Homo sapiens  | 90.78             | 8.36E-10     | 11500000     | PRDXI_HUMAN      | OS=Homo sapiens  | 90.78        |
| Q00159     | MYO1C_HUMAN  | OS=Homo sapiens  | 85.90             | 2.57E-09     | 16500000     | MYO1C_HUMAN      | OS=Homo sapiens  | 85.90        |
| P68104     | EF1A1_HUMAN  | OS=Homo sapiens  | 83.73             | 4.24E-09     | 19500000     | EF1A1_HUMAN      | OS=Homo sapiens  | 83.73        |
| Q05639     | EF1A2_HUMAN  | OS=Homo sapiens  | 83.73             | 4.24E-09     | 19500000     | EF1A2_HUMAN      | OS=Homo sapiens  | 83.73        |
| Q5VTE0     | EF1A3_HUMAN  | OS=Homo sapiens  | 83.73             | 4.24E-09     | 19500000     | EF1A3_HUMAN      | OS=Homo sapiens  | 83.73        |
| P15328     | FOLR2_HUMAN  | OS=Homo sapiens  | 80.60             | 8.71E-09     | 25400000     | FOLR2_HUMAN      | OS=Homo sapiens  | 80.60        |
| Q69KHE     | CD109_HUMAN  | OS=Homo sapiens  | 74.04             | 3.94E-08     | 68600000     | CD109_HUMAN      | OS=Homo sapiens  | 74.04        |
| P29401     | TKT_HUMAN    | OS=Homo sapiens  | 71.68             | 6.79E-08     | 25400000     | TKT_HUMAN        | OS=Homo sapiens  | 71.68        |

Table S1. Proteomic profiles of microvesicles from PV- and mock- infected cells at 8 hpi.
We report the full proteomic profile of microvesicles from PV-infected cells carry 65 identified host protein matches (columns to the left of the green column, including Accession number, protein name, -10lgP, P, and aggregated area from one of the experiments). Microvesicles from mock-infected cells carry five host protein matches (columns to the right of the green column). All proteins identified in samples from mock-infected cells were also identified in samples from PV-infected cells. All proteomic analyses were conducted at a specified precursor ion (MS1) error tolerance of 10 ppm, a fragment ion (MS/MS) error tolerance of 0.02 Da. and a target-decoy false discovery threshold of 0.1%.