

Virulence from vesicles: Novel mechanisms of host cell injury

by *Escherichia coli* O104:H4 outbreak strain

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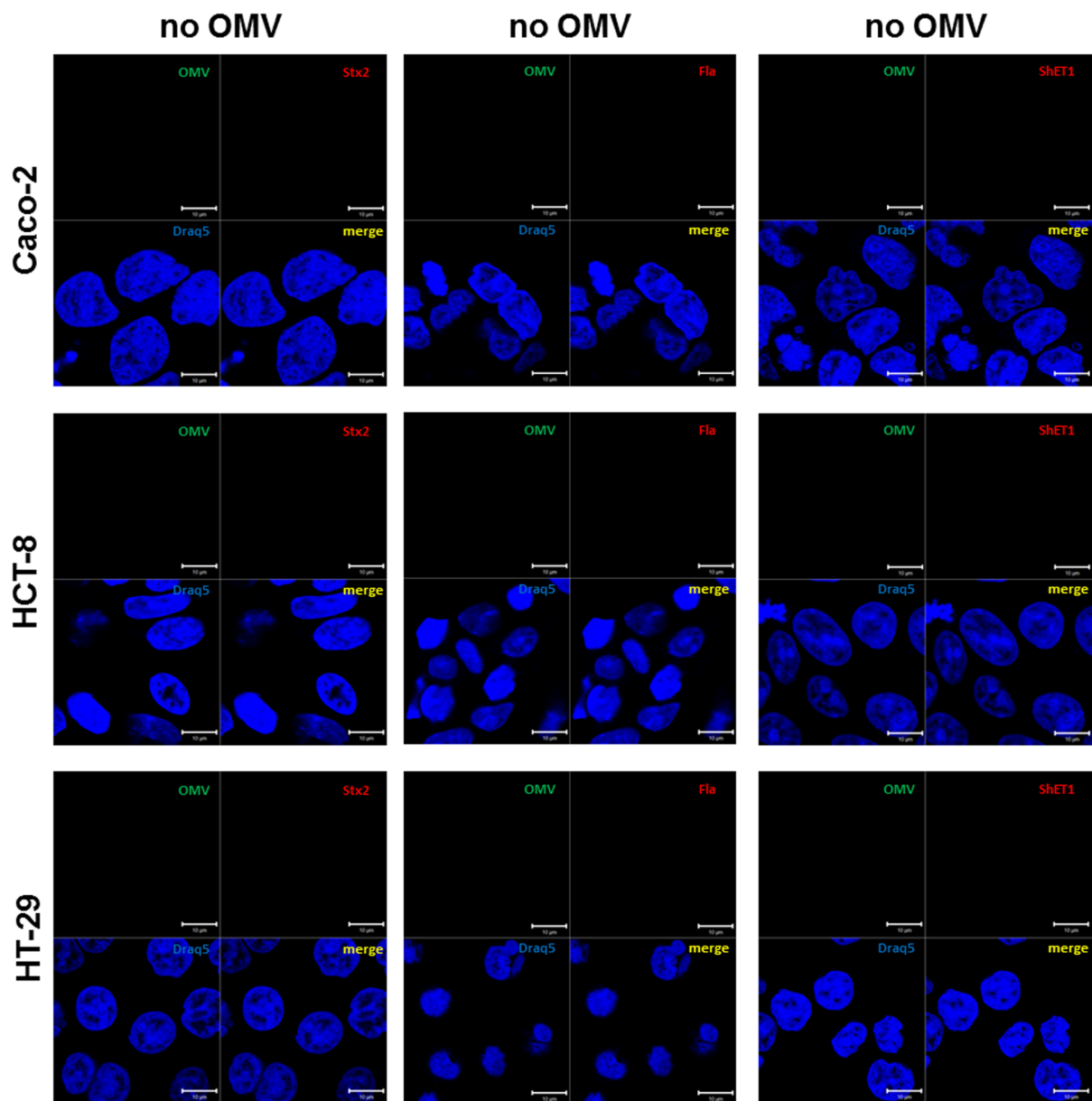
Supplementary Methods

Comparison of genome sequences of *E. coli* O104:H4 outbreak isolates. To confirm that the *E. coli* O104:H4 strain LB226692 and the *stx*-negative strain C227-11Φcu are highly homologous (“isogenic”) and representative for the large outbreak in 2011, we determined the genome sequences of both strains using Illumina paired-end sequencing technology¹. After *denovo* assembly as described elsewhere¹, we extracted - using STEC O157:H7 strain Sakai as name-giving reference sequence (GenBank accession no. NC_002695) - all coding regions of both strains and compared them to the fully sequenced and finished genome sequence of *E. coli* O104:H4 outbreak strain 2011C-3493² (GenBank accession no. CP003289). Overall, 3533 genes were completely present in all three *E. coli* O104:H4 strains (Supplementary Table S6). Among these, only three genes (ECs0625, ECs0972, and ECs1721) contained single nucleotide polymorphisms (SNP) in comparison to strain 2011C-3493: in ECs0625 there was a SNP in LB226692 (nucleotide position 2650, C→A; Gln→Lys), in ECs0972 there was a SNP in C227-11Φcu (nucleotide position 948, C→G; Gly→Arg), and in ECs1721 there was a SNP in C227-11Φcu (nucleotide position 848, T→A; Glu→Val). Raw reads of strains C227-11Φcu and LB226692 are available at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena/>) under the study accession number PRJEB9313.

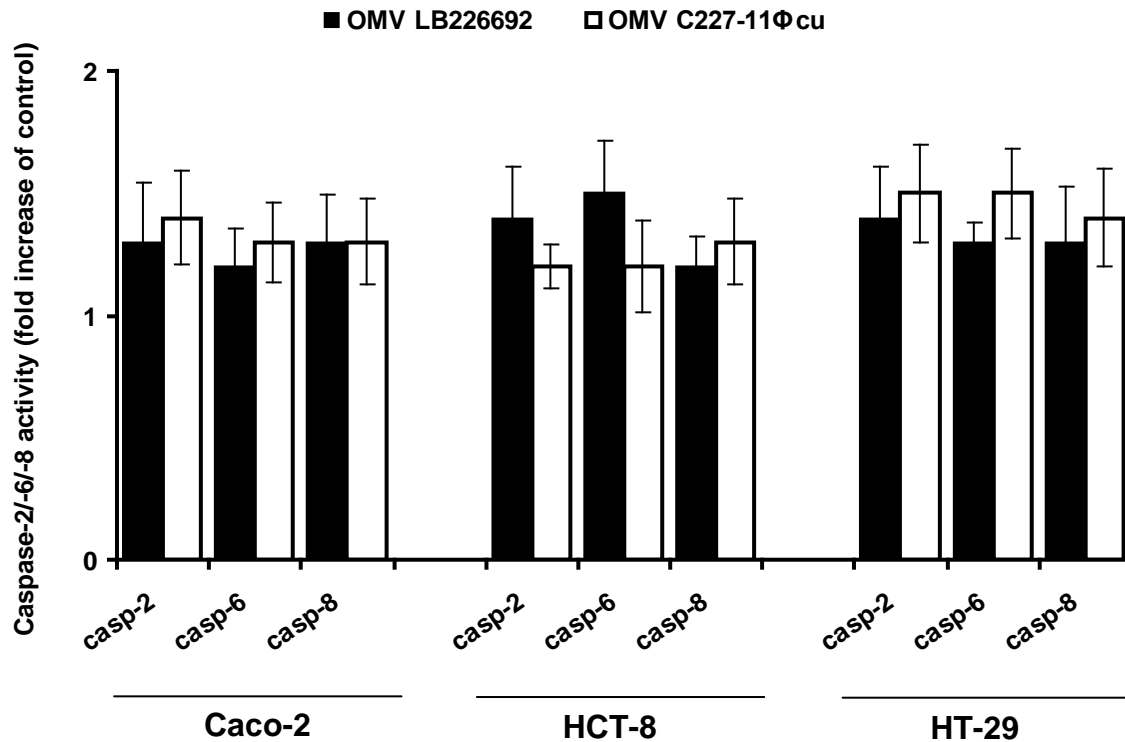
Supplementary references

1. Leopold, S. R., Goering, R. V., Witten, A., Harmsen, D. & Mellmann, A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J. Clin. Microbiol.* **52**, 2365-2370 (2014).
2. Ahmed, S. A. *et al.* Genomic comparison of *Escherichia coli* O104:H4 isolates from 2009 and 2011 reveals plasmid, and prophage heterogeneity, including Shiga toxin encoding phage *stx2*. *PLoS One* **7**, e48228 (2012).

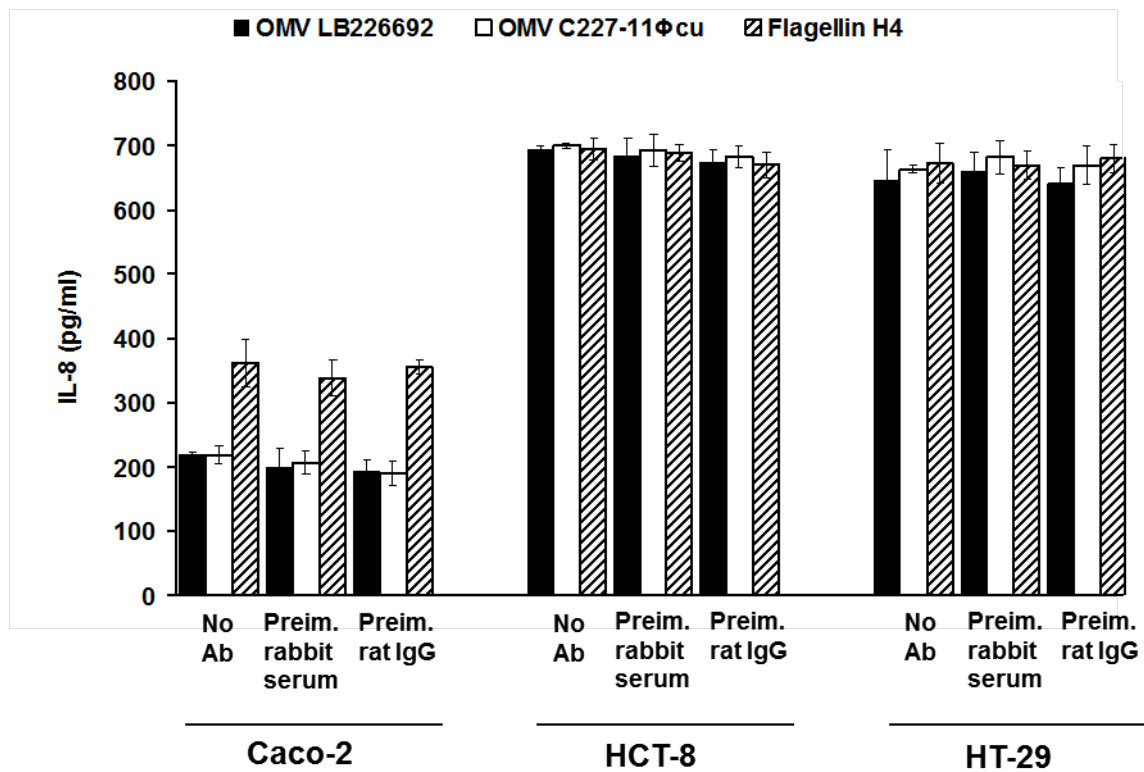
Supplementary Figures



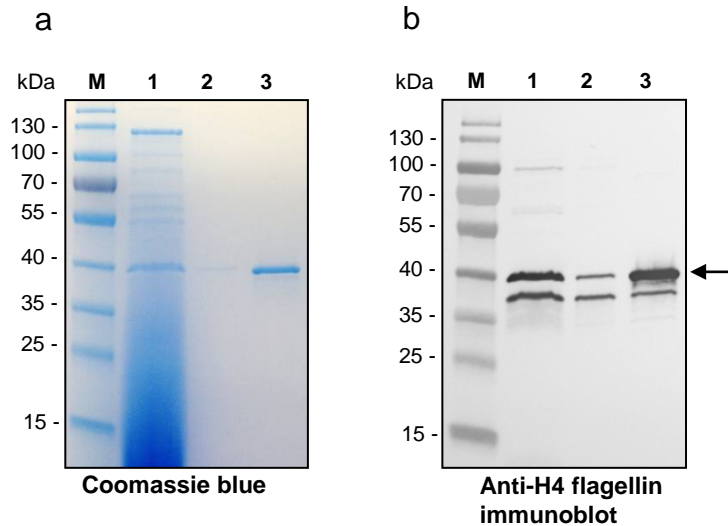
Supplementary Figure S1. CLSM of control cells. Cells were incubated for 6 h with OMV buffer (20 mM TRIS-HCl, pH 8.0) instead of OMVs and stained for OMVs, Stx2a, H4 flagellin (Fla), ShET1 and nuclei as indicated in the legend to Fig. 5 b-d. Pictures were taken using a laser-scanning microscope (LSM 510 META microscope, equipped with a Plan-Apochromat 63x/1.4 oil immersion objective). Single fluorescence channels and merged images are shown as indicated. Scale bars are 10 μm.



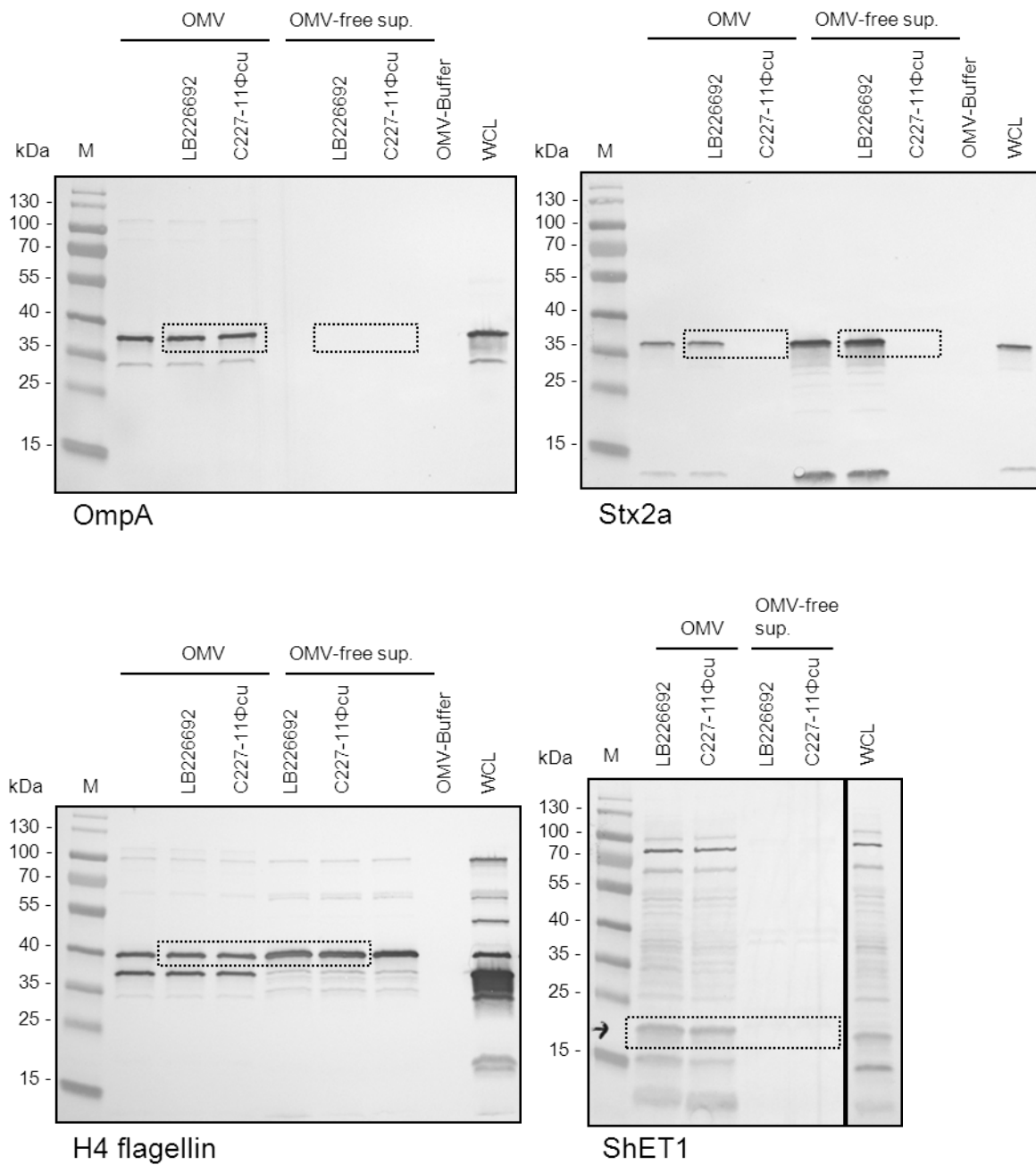
Supplementary Figure S2. OMVs LB226692 do not activate caspase-2, caspase-6, and caspase-8 in human intestinal epithelial cells. Cells were incubated (48 h) with OMVs LB226692 (containing 580 ng/ml of Stx2a) or OMVs C227-11Φcu (Stx2a-negative) or remained untreated. Caspase-2, caspase-6, and caspase-8 activities in cell lysates were determined using colorimetric substrates (VDVAD-pNA, VEID-pNA, and IETD-pNA, respectively) (Caspase Colorimetric Protease Assay Sampler Kit, Invitrogen). The colour intensity, which is proportional to the level of caspase enzymatic activity, was measured spectrophotometrically at 405 nm (FLUOstar OPTIMA reader). The caspase activity in OMV-treated cells was expressed as a fold-increase of that in untreated control cells (set up as 1). Data are means \pm standard deviations from three independent experiments.



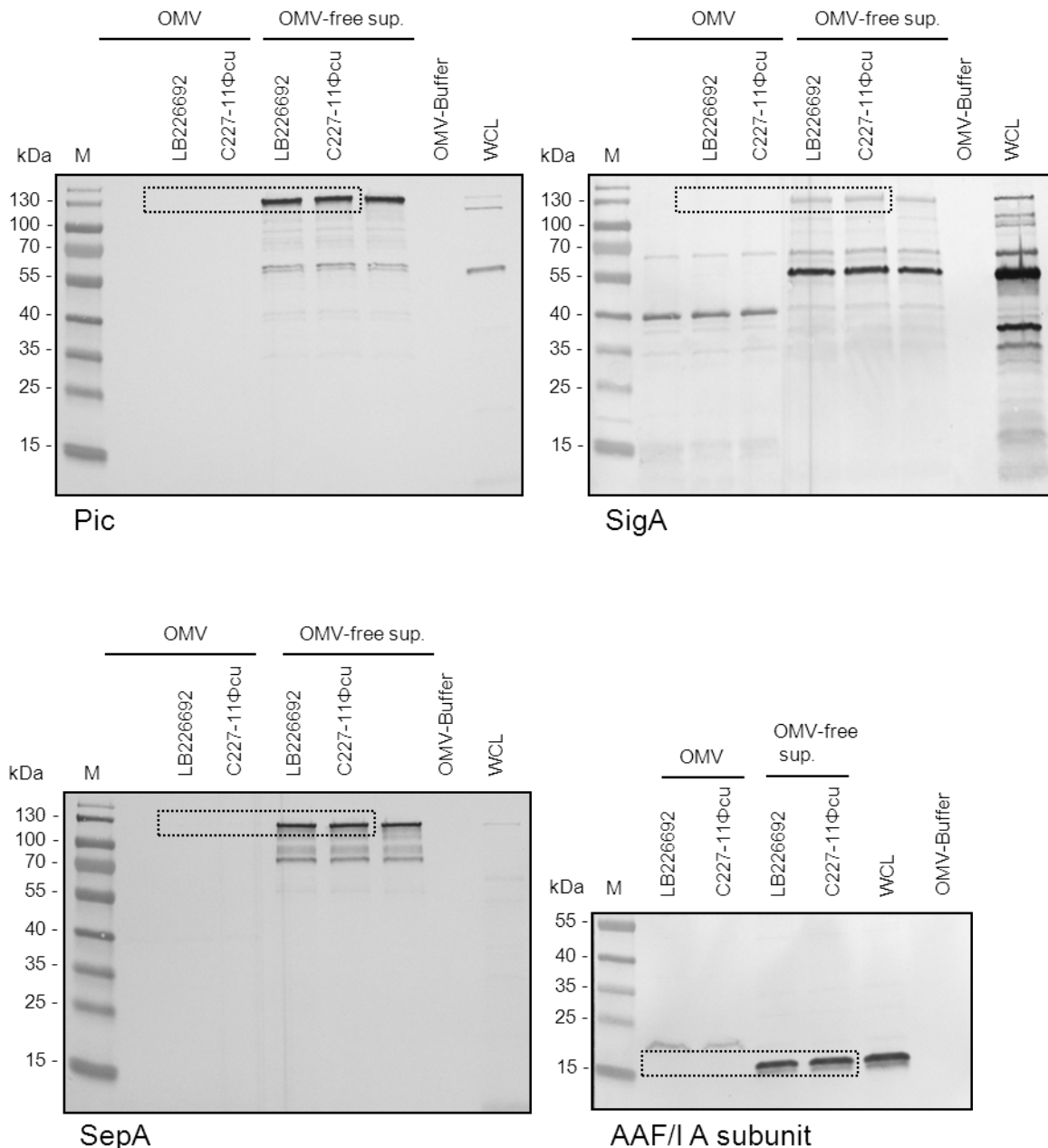
Supplementary Figure S3. Preimmune sera do not inhibit IL-8 secretion elicited by *E. coli* O104:H4 OMVs and flagellin from human intestinal epithelial cells. Cells were exposed for 24 h to: OMVs or isolated H4 flagellin in the absence of sera (columns No Ab); OMVs or flagellin which had been preincubated with preimmune rabbit serum (control to the anti-H4 antibody); OMVs or flagellin after the cell preincubation with preimmune rat IgG (control the anti-TLR5 neutralizing antibody). IL-8 secretion was quantified using a Single-Analyte IL-8 ELISArray. Data are means \pm standard deviations from three independent experiments.



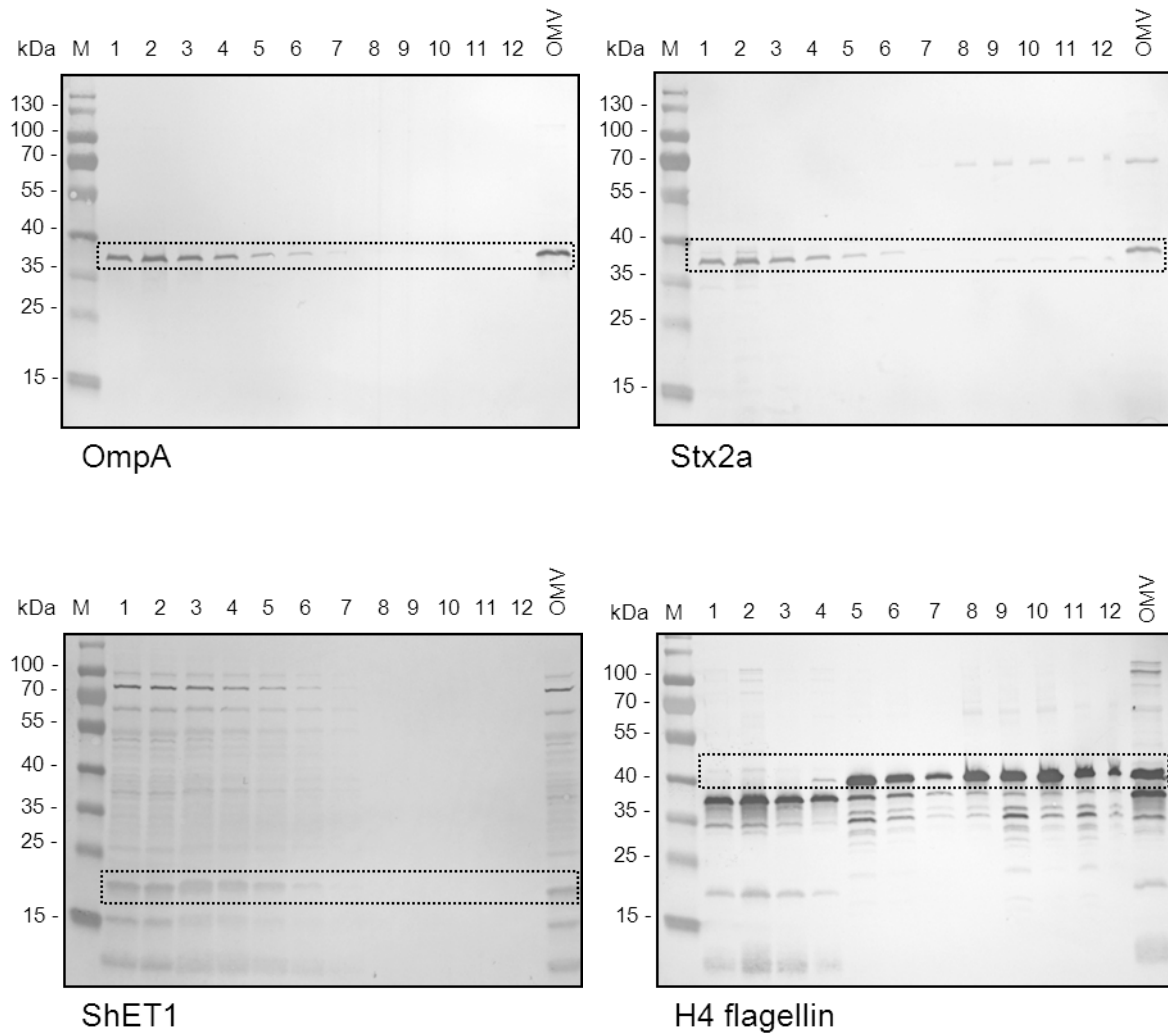
Supplementary Figure S4. Isolation of H4 flagellin from *E. coli* O104:H4 strain C227-11Φcu. H4 flagellin isolated from strain C227-11Φcu (~5 μg of protein/lane) (lanes 3) was (a) separated by SDS-PAGE and the gel was stained with Coomassie blue. (b) The proteins were transferred to a polyvinylidene fluoride membrane and immunoblotted with anti-H4 antibody. 50-fold concentrated OMV-free supernatant of strain C227-11Φcu (lanes 1) and 50-fold concentrated C227-11Φcu OMVs (lanes 2) were included for comparison. M, Marker IV (PepLab Biotechnologie); the sizes of the bands are shown on the left side. The arrow in panel b depicts the ~40 kDa flagellin band which is recognized by the anti-H4 antibody.



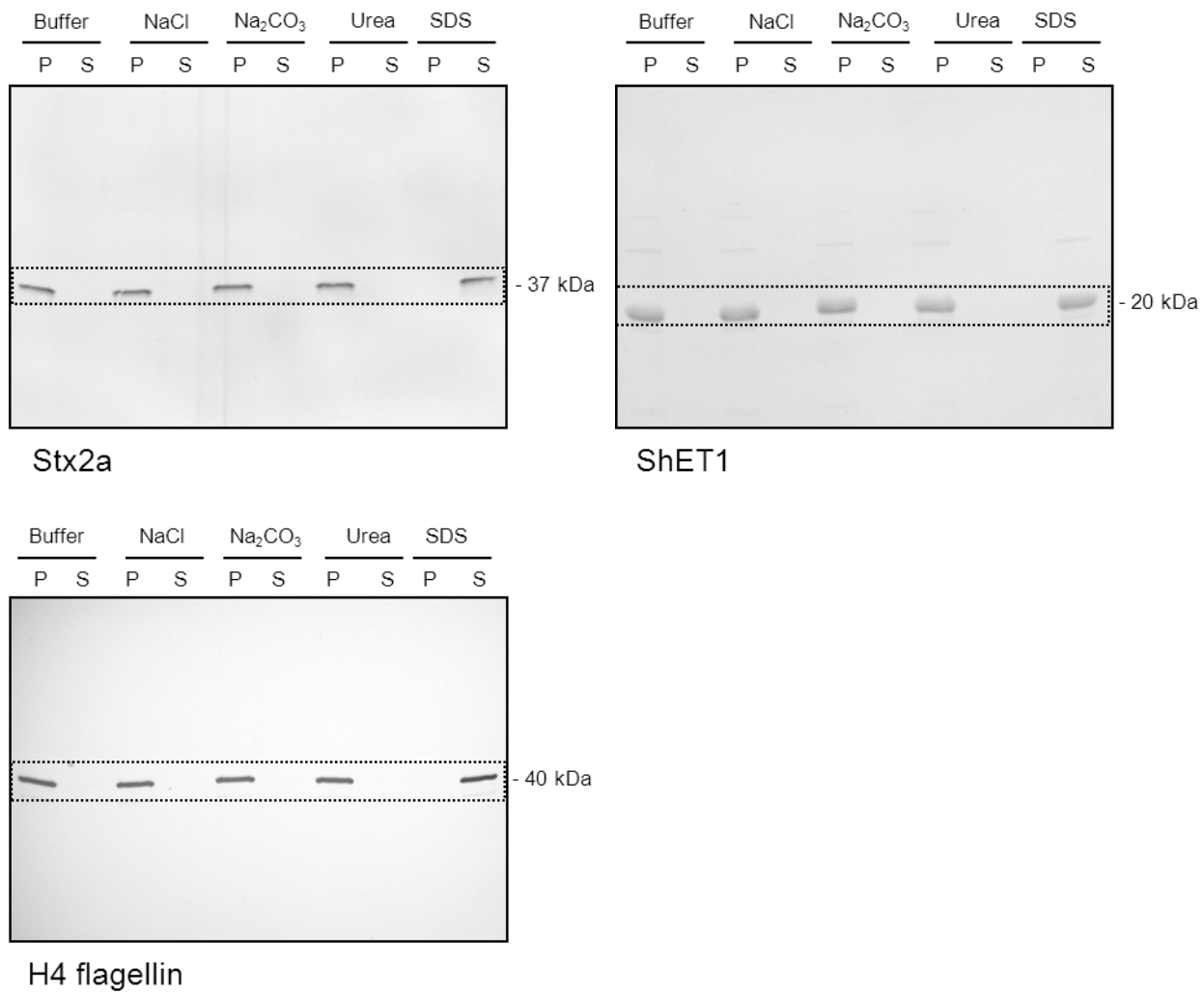
Supplementary Figure S5 (part 1) - continued on the next page



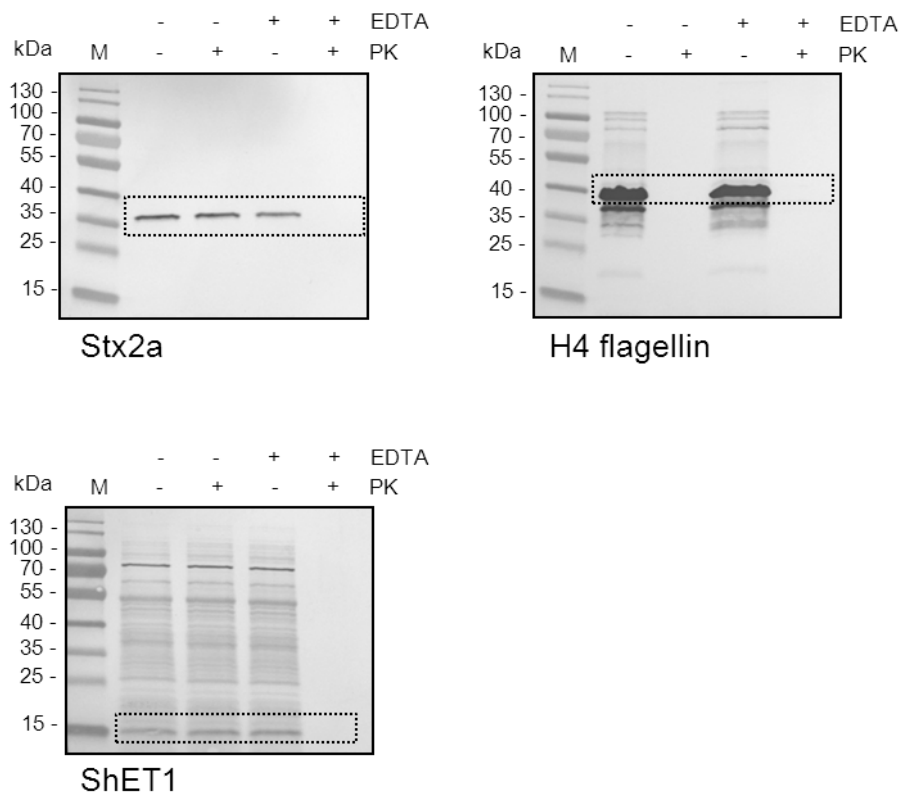
Supplementary Figure S5. Distribution of virulence factors of *E. coli* O104:H4 outbreak strain in OMVs and OMV-free supernatants. Full immunoblots from which the cropped versions shown in Fig. 2a were created. The crops shown in Fig. 2a are indicated by boxes. Whole cell lysate (WCL) and OMV-buffer were used as a positive and negative control, respectively. M, Marker IV (Peqlab Biotechnologie); the sizes of the bands are shown on the left side. (The bands without an indication depict an additional Stx2a-positive outbreak isolate which was initially investigated as a control but was not included in the final analysis).



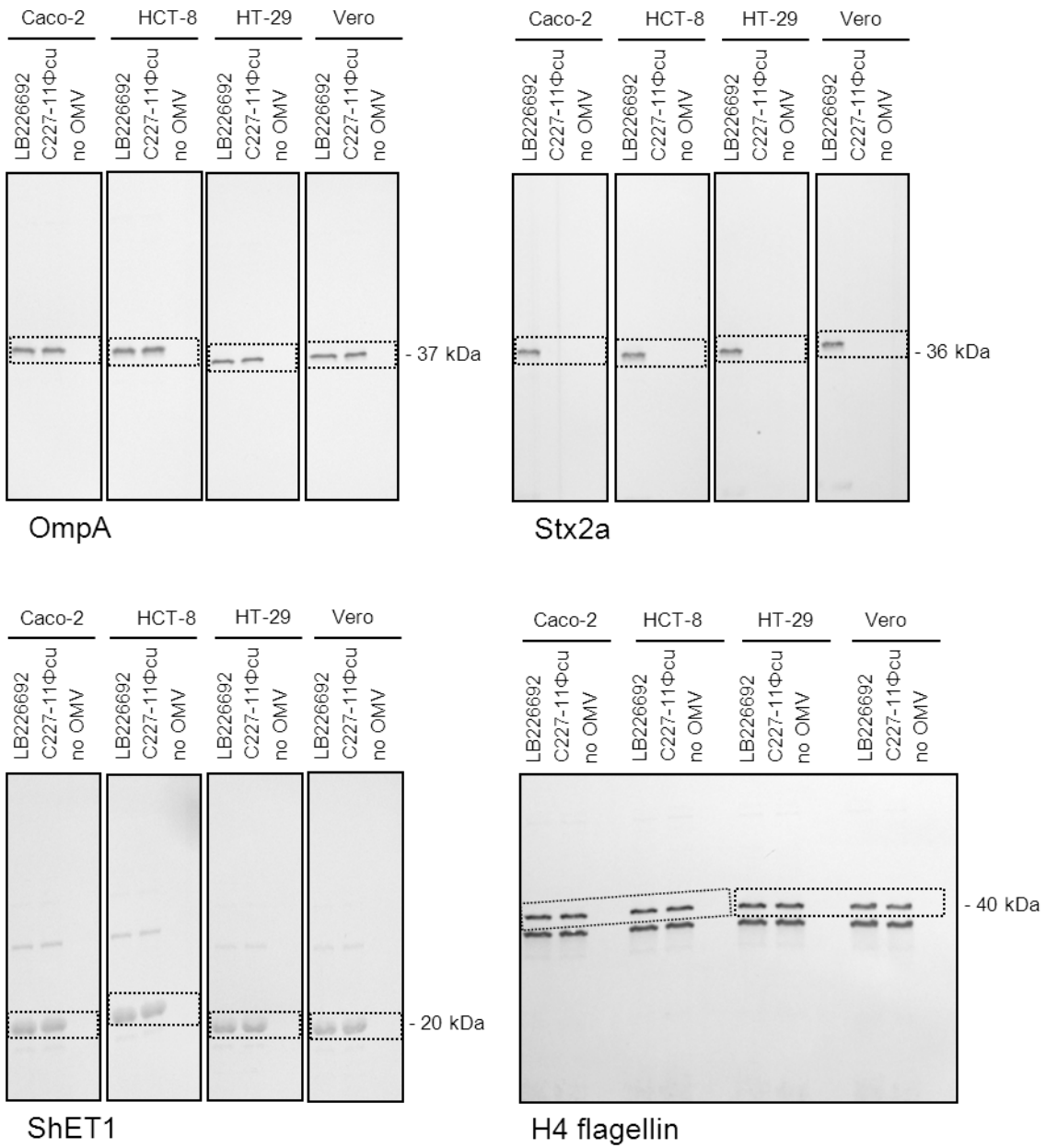
Supplementary Figure S6. Distribution of Stx2a, ShET1 and H4 flagellin in OptiPrep density gradient fractions of OMVs LB226692. Full immunoblots corresponding to the cropped versions shown in Fig. 2b are shown. The crops shown in Fig. 2b are indicated by boxes. The numbers above the blots indicate the order of the fractions in which they were collected from top to bottom after the gradient ultracentrifugation. The lanes designated OMV contain non-fractionated OMVs (positive control). M, Marker IV (PepLab Biotechnologie); the sizes of the bands are shown on the left side.



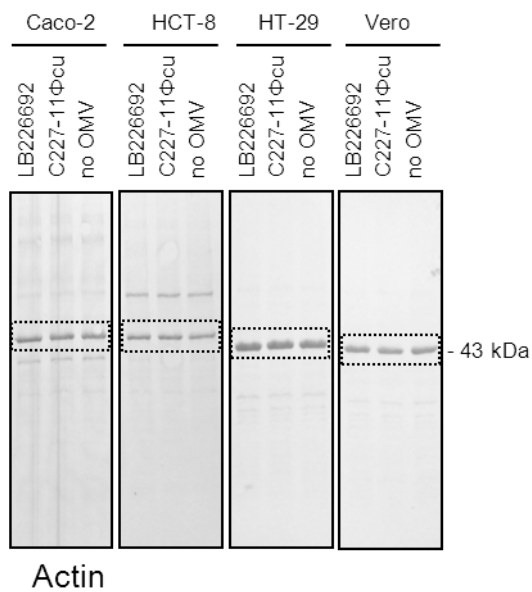
Supplementary Figure S7. Dissociation assay of purified OMVs LB226692. Full immunoblots corresponding to the cropped versions shown in Fig. 2c are shown. The crops shown in Fig. 2c are indicated by boxes. The sizes of the immunoreactive bands are shown on the right side.



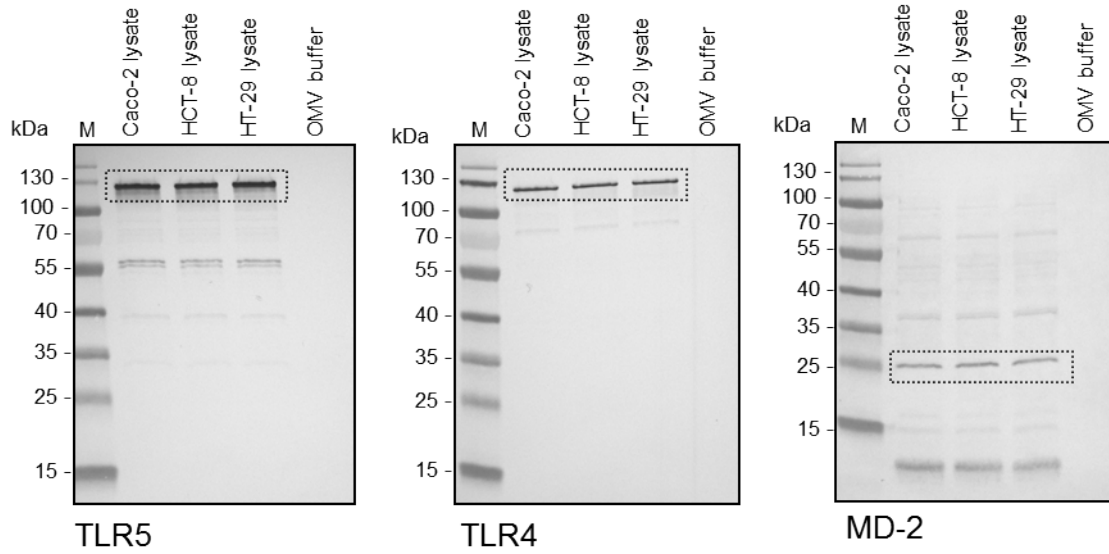
Supplementary Figure S8. Proteinase K assay of LB226692 OMVs. Full immunoblots corresponding to the cropped versions shown in Fig. 3c are shown. The crops shown in Fig. 3c are indicated by boxes. The corresponding treatments with proteinase K (PK) (untreated, PK-; treated; PK+) and EDTA (EDTA-, intact OMVs; EDTA+, lysed OMVs) are indicated above the immunoblots. M, Marker IV (Peqlab Biotechnologie); the sizes of the bands are shown on the left side.



Supplementary Figure S9 (part 1) - continued on the next page



Supplementary Figure S9. OMV-mediated intracellular delivery of Stx2a, ShET1, and H4 flagellin into human intestinal epithelial cells and Vero cells. Full immunoblots corresponding to the cropped versions shown in Fig. 5a are shown. The crops shown in Fig. 5a are indicated by boxes. The sizes of the immunoreactive bands are shown on the right side.



Supplementary Figure S10. Detection of TLR5, TLR4 and MD-2 in lysates of Caco-2, HCT-8, and HT-29 cells using immunoblot. Full immunoblots corresponding to the cropped versions shown in Fig. 8e are shown. The crops shown in Fig. 8e are indicated by boxes. OMV buffer served as a negative control. M, Marker IV (PeproLab Biotechnologie); the sizes of the bands are shown on the left side.

Supplementary Tables

Supplementary Table S1. DNA content in *E. coli* O104:H4 OMVs

OMVs from strain	DNA amount (ng/ml) ^a in OMVs processed as indicated		
	Intact	Intact	Lysed after
	DNase-untreated	DNase-treated	DNase treatment
LB226692	107.2 ± 15.5	105.2 ± 16.3	100.8 ± 13.6
C227-11Φ _{cu}	103.3 ± 19.7	101.1 ± 19.6	99.4 ± 17.5

^a The DNA amounts are expressed as means ± standard deviations from measurements of three different batches of each OMV preparation.

Supplementary Table S2. Virulence genes of *E. coli* O104:H4 outbreak strain detected in OMVs

Virulence locus	Product/marker for ^a	LB226692 OMVs		C227-11Φcu OMVs	
		DNase No ^b	DNase Yes ^c	DNase No ^b	DNase Yes ^c
<i>stx_{2a}A</i>	Stx2a A subunit	+/+	+/+	-/-	-/-
<i>stx_{2a}B</i>	Stx2a B subunit	+/+	+/+	-/-	-/-
<i>iha</i>	Iha	+/-	-/-	+/-	-/-
<i>ter</i> ZABCDEF cluster	Tellurite resistance	+/+	+/+	+/+	+/+
<i>pic</i>	Pic	+/-	-/-	+/-	-/-
<i>sigA</i>	SigA	+/-	-/-	+/-	-/-
<i>set1A</i>	ShET1 A subunit	+/-	-/-	+/-	-/-
<i>set1B</i>	ShET1 B subunit	+/-	-/-	+/-	-/-
<i>fliC_{H4}</i>	H4 flagellin biosynthetic cluster	+/-	-/-	+/-	-/-
<i>aggR</i>	AggR	+/-	-/-	+/-	-/-
<i>aggA</i>	AAF/I fimbriae major subunit	+/-	-/-	+/-	-/-
<i>sepA</i>	SepA	+/-	-/-	+/-	-/-
<i>aap</i>	Dispersin	+/-	-/-	+/-	-/-
<i>aatA</i>	Dispersin transporter	+/-	-/-	+/-	-/-

^a Stx2a, Shiga toxin 2a (A and B subunit); Iha, iron-regulated gene A homologue adhesin; Pic, protein involved in intestinal colonisation; SigA, *Shigella* IgA protease-like homologue; ShET1, *Shigella* enterotoxin 1 (A and B subunit); AggR, AggR transcriptional regulator; SepA, *Shigella* extracellular protein A.

^b Presence (+) or absence (-) of amplicon in non-purified OMVs / in density gradient-purified OMVs.

^c Presence (+) or absence (-) of amplicon in DNase-treated intact OMVs / DNase-treated and subsequently lysed OMVs.

Supplementary Table S3. Proteins identified in OMVs from *E. coli* O104:H4 outbreak strain

Accession no.	Protein description	OMV LB226692	OMV C227-11Φcu
Outer membrane			
gi 354857941	Outer membrane protein A	+ ^a	+
gi 341916745	Outer membrane protein C	+	+
gi 341920353	Outer membrane protein F	+	+
gi 472423767	Outer membrane protein W	+	+
gi 354857797	Outer membrane protein X	+	+
gi 130682	Outer membrane porin protein LC	+	+
gi 354863302	Outer membrane protein Slp	+	+
gi 354863719	Outer membrane protein TolC	+	+
gi 354869769	Outer membrane lipoprotein SlyB	+	+
gi 354857669	Peptidoglycan-associated lipoprotein	+	+
gi 354869804	Major outer membrane lipoprotein	+	+
gi 341918794	Murein lipoprotein lpp	+	+
gi 354867469	Long-chain fatty acid transporter	+	+
gi 341917536	Hypothetical protein C22711_1180	+	+
gi 341919927	Hypothetical protein C22711_3567	+	+
gi 341918829	Hypothetical protein C22711_2472	+	+
gi 341917258	Hypothetical protein C22711_0902	+	+
gi 341917385	Hypothetical protein C22711_1030	+	+
gi 341916624	Hypothetical protein C22711_0268	+	+
Periplasm			
gi 340736504	Shiga toxin 2 A subunit	+	-
gi 340736503	Shiga toxin 2 B subunit	+	-
gi 354867049	Flagellar hook-basal body complex protein FliE	+	+
gi 354860155	Hypothetical protein EUAG_00762	-	+
gi 341916992	Hypothetical protein C22711_0636	+	+
Inner membrane			
gi 354859437	Entericidin B	+	+
gi 354870404	Glutamate:gamma-aminobutyrate antiporter	+	-
gi 354857660	Cytochrome d ubiquinol oxidase subunit1	+	+
gi 354863369	Xylose import ATP-binding protein XylG	+	+
gi 341920553	Hypothetical protein C22711_4192	+	+
gi 341917249	Hypothetical protein C22711_0893	+	+
gi 110342306	Hypothetical protein ECP_0514	+	+
Cytoplasm			
gi 150957079	Bacterioferrin	+	+
gi 26108577	Ferritin 1	+	+
gi 816212539	60 kDa chaperonin	+	+
gi 218355112	Pyruvate dehydrogenase	+	+
gi 938166	Glutamate decarboxylase	+	+
gi 354867389	O-succinylbenzoate synthase	+	+
gi 354867171	UDP-glucose 6-dehydrogenase	+	+
gi 354861355	Glucose-6-phosphate isomerase	+	+

gi 340737814	Glutamine synthetase	+	+
gi 354857287	Flagellar brake protein YcgR	+	+
gi 354863851	Translation initiation factor IF-2	+	-
gi 1658028	Dehydrin	-	+
gi 446318982	Phage head protein, partial	+	-
gi 384221	Starvation-inducible DNA-binding protein	+	+
gi 745374501	50S ribosomal subunit protein L1	+	+
gi 816212730	50S Ribosomal protein L10	+	+
gi 340738332	50S ribosomal protein L13	+	+
gi 340742056	30S ribosomal protein S2	+	+
gi 340738394	30S ribosomal protein S4	+	+
gi 354858002	Hypothetical protein EUAG_01346	+	-
gi 354858035	Hypothetical protein EUAG_01379	+	+
gi 341918492	Hypothetical protein C22711_2135	+	+
Extracellular			
gi 354867035	Flagellin H4	+	+
gi 341920310	Hypothetical protein C22711_3950	+	+
Unknown localisation			
gi 341918676	DNA-binding transcriptional activator OsmE	+	+
gi 354868859	Osmotically-inducible lipoprotein E	+	+
gi 354869861	P2 family phage major capsid protein	+	-
gi 340739578	fructose-bisphosphate aldolase	+	+
gi 189357744	Conserved hypothetical protein	+	+
gi 4585432	Hypothetical protein phage	+	-
gi 209762284	Hypothetical protein ECs3533	+	+
gi 341918457	Hypothetical protein C22711_2100	+	+
gi 354856238	Hypothetical protein EUAG_05098	+	+
gi 354858036	Hypothetical protein EUAG_01380	+	+
gi 341918458	Hypothetical protein C22711_2101	+	+
gi 354868604	Hypothetical protein EUAG_05105	+	+
gi 354857849	Hypothetical protein EUAG_01193	+	+
gi 341917439	Hypothetical protein C22711_1083	+	+
gi 354869668	Hypothetical protein EUAG_04529	+	+
gi 341918933	Hypothetical protein C22711_2576	+	+
gi 354863782	Hypothetical protein EUAG_01956	+	+
gi 341919203	Hypothetical protein C22711_2845	+	+
gi 354869668	Hypothetical protein EUAG_04529	+	+
gi 341918444	Hypothetical protein C22711_2087	+	+
gi 341918945	Hypothetical protein C22711_2588	+	+
gi 354857030	Hypothetical protein EUAG_01390	+	+

^a +, presence of the protein; -, absence of the protein.

Supplementary Table S4. Concentrations^a of the total protein, Stx2a, H4 flagellin and O104 LPS in purified *E. coli* O104:H4 OMVs

OMVs from strain	Total protein concentration ($\mu\text{g/ml}$)	Stx2a concentration ($\mu\text{g/ml}$)	Flagellin H4 concentration ($\mu\text{g/ml}$)	LPS O104 concentration ($\mu\text{g/ml}$)
LB226692	471 \pm 53.6	58 \pm 4.5	80 \pm 5.1	950 \pm 69.7
C227-11 Φ cu	466 \pm 42.1	0	79 \pm 4.4	946 \pm 54.2

^a Expressed as means \pm standard deviations from measurements of five different batches of each OMV preparation.

Supplementary Table S5. Cytotoxicity titres of *E. coli* O104:H4 OMVs on human intestinal epithelial cells and Vero cells

Cell line	Cytotoxicity titres ^a			
	OMV LB226692	OMV LB226692 (with anti-Stx2a antibody) ^b	OMV C227-11Φcu	EDL933 supernatant (positive control)
Caco-2	8 (8-16) ^c	8 (4-32) ^c	< 2	8 (8-16) ^c
HCT-8	32 (16-64) ^c	32 (16-64) ^c	< 2	32 (16-128) ^c
HT-29	32 (16-128) ^c	32 (16-128) ^c	< 2	32 (16-64) ^c
Vero	3072 (2048-4096)	3072 (2048-8192)	< 2	4096 (1024-8192)

^a Reciprocal values of the highest sample dilutions that killed 50% cells after 72 h; shown as a median (range) from five independent experiments; <2, no cytotoxicity observed with samples diluted 1:2.

^b OMVs were preincubated with anti-Stx2a neutralizing antibody before adding to cells.

^c $P < 0.01$ (one-way ANOVA) for the cytotoxicity titre on each intestinal epithelial line compared to that on Vero cells.

Supplementary Table S6. Genes present in all three *E. coli* O104:H4 strains whose genome sequences were compared (gene names are locus tags from the *E. coli* O157:H7 reference strain Sakai (GenBank accession no. NC_002695))

ECs0002	ECs0596	ECs1416	ECs2407	ECs3111	ECs3656	ECs4180	ECs4790
ECs0003	ECs0597	ECs1417	ECs2408	ECs3112	ECs3657	ECs4181	ECs4791
ECs0004	ECs0598	ECs1419	ECs2409	ECs3113	ECs3658	ECs4182	ECs4792
ECs0005	ECs0599	ECs1420	ECs2410	ECs3114	ECs3659	ECs4183	ECs4793
ECs0006	ECs0600	ECs1421	ECs2411	ECs3115	ECs3660	ECs4184	ECs4794
ECs0007	ECs0601	ECs1423	ECs2412	ECs3117	ECs3661	ECs4185	ECs4795
ECs0008	ECs0606	ECs1424	ECs2413	ECs3118	ECs3662	ECs4186	ECs4796
ECs0009	ECs0608	ECs1425	ECs2414	ECs3119	ECs3663	ECs4187	ECs4808
ECs0010	ECs0609	ECs1426	ECs2415	ECs3120	ECs3664	ECs4188	ECs4809
ECs0012	ECs0610	ECs1427	ECs2416	ECs3124	ECs3665	ECs4189	ECs4810
ECs0013	ECs0611	ECs1428	ECs2417	ECs3125	ECs3666	ECs4191	ECs4811
ECs0014	ECs0612	ECs1429	ECs2418	ECs3126	ECs3667	ECs4192	ECs4814
ECs0015	ECs0613	ECs1431	ECs2419	ECs3127	ECs3668	ECs4193	ECs4815
ECs0017	ECs0614	ECs1432	ECs2420	ECs3128	ECs3669	ECs4194	ECs4816
ECs0018	ECs0615	ECs1433	ECs2421	ECs3137	ECs3670	ECs4195	ECs4817
ECs0019	ECs0616	ECs1434	ECs2423	ECs3138	ECs3671	ECs4196	ECs4818
ECs0026	ECs0617	ECs1435	ECs2424	ECs3139	ECs3672	ECs4197	ECs4819
ECs0027	ECs0618	ECs1436	ECs2425	ECs3140	ECs3673	ECs4198	ECs4821
ECs0028	ECs0619	ECs1437	ECs2426	ECs3141	ECs3674	ECs4199	ECs4823
ECs0029	ECs0622	ECs1438	ECs2427	ECs3142	ECs3675	ECs4200	ECs4824
ECs0030	ECs0623	ECs1440	ECs2428	ECs3143	ECs3676	ECs4201	ECs4825
ECs0031	ECs0624	ECs1441	ECs2429	ECs3144	ECs3677	ECs4202	ECs4826
ECs0032	ECs0625	ECs1442	ECs2430	ECs3145	ECs3678	ECs4203	ECs4828
ECs0033	ECs0626	ECs1443	ECs2431	ECs3146	ECs3679	ECs4204	ECs4829
ECs0034	ECs0627	ECs1444	ECs2432	ECs3147	ECs3680	ECs4205	ECs4830
ECs0035	ECs0628	ECs1445	ECs2433	ECs3148	ECs3681	ECs4206	ECs4831
ECs0036	ECs0629	ECs1446	ECs2434	ECs3149	ECs3682	ECs4207	ECs4832
ECs0037	ECs0630	ECs1447	ECs2435	ECs3150	ECs3683	ECs4208	ECs4833
ECs0038	ECs0631	ECs1448	ECs2437	ECs3151	ECs3684	ECs4209	ECs4834
ECs0039	ECs0632	ECs1449	ECs2438	ECs3152	ECs3685	ECs4210	ECs4835
ECs0040	ECs0633	ECs1450	ECs2439	ECs3153	ECs3686	ECs4211	ECs4836
ECs0041	ECs0634	ECs1451	ECs2440	ECs3154	ECs3687	ECs4212	ECs4837
ECs0042	ECs0635	ECs1452	ECs2441	ECs3155	ECs3688	ECs4213	ECs4838
ECs0043	ECs0636	ECs1453	ECs2442	ECs3156	ECs3689	ECs4214	ECs4839
ECs0044	ECs0637	ECs1454	ECs2443	ECs3159	ECs3690	ECs4215	ECs4840
ECs0045	ECs0638	ECs1455	ECs2444	ECs3160	ECs3691	ECs4216	ECs4841
ECs0046	ECs0639	ECs1456	ECs2445	ECs3161	ECs3692	ECs4217	ECs4842
ECs0047	ECs0640	ECs1457	ECs2446	ECs3162	ECs3693	ECs4218	ECs4843
ECs0048	ECs0641	ECs1458	ECs2447	ECs3163	ECs3694	ECs4219	ECs4844
ECs0049	ECs0642	ECs1459	ECs2448	ECs3164	ECs3695	ECs4220	ECs4845
ECs0050	ECs0643	ECs1460	ECs2449	ECs3165	ECs3696	ECs4221	ECs4846
ECs0051	ECs0644	ECs1461	ECs2450	ECs3166	ECs3697	ECs4222	ECs4847
ECs0054	ECs0645	ECs1462	ECs2451	ECs3167	ECs3698	ECs4223	ECs4848
ECs0055	ECs0646	ECs1463	ECs2452	ECs3168	ECs3699	ECs4224	ECs4849
ECs0056	ECs0647	ECs1464	ECs2453	ECs3169	ECs3700	ECs4225	ECs4850
ECs0057	ECs0648	ECs1465	ECs2454	ECs3170	ECs3701	ECs4226	ECs4851
ECs0058	ECs0649	ECs1466	ECs2455	ECs3171	ECs3702	ECs4227	ECs4852

ECs0059	ECs0650	ECs1467	ECs2456	ECs3172	ECs3703	ECs4228	ECs4853
ECs0060	ECs0651	ECs1468	ECs2457	ECs3173	ECs3704	ECs4229	ECs4856
ECs0062	ECs0652	ECs1469	ECs2458	ECs3174	ECs3706	ECs4230	ECs4857
ECs0063	ECs0653	ECs1470	ECs2459	ECs3175	ECs3708	ECs4231	ECs4858
ECs0064	ECs0654	ECs1471	ECs2460	ECs3176	ECs3709	ECs4232	ECs4859
ECs0065	ECs0655	ECs1472	ECs2461	ECs3177	ECs3710	ECs4233	ECs4860
ECs0066	ECs0656	ECs1473	ECs2462	ECs3178	ECs3712	ECs4234	ECs4861
ECs0067	ECs0657	ECs1474	ECs2463	ECs3180	ECs3713	ECs4235	ECs4862
ECs0068	ECs0658	ECs1475	ECs2464	ECs3181	ECs3715	ECs4236	ECs4863
ECs0069	ECs0659	ECs1476	ECs2465	ECs3182	ECs3716	ECs4238	ECs4865
ECs0070	ECs0660	ECs1477	ECs2466	ECs3183	ECs3719	ECs4239	ECs4867
ECs0071	ECs0661	ECs1478	ECs2467	ECs3184	ECs3720	ECs4240	ECs4868
ECs0072	ECs0662	ECs1479	ECs2468	ECs3185	ECs3724	ECs4241	ECs4869
ECs0074	ECs0663	ECs1480	ECs2469	ECs3186	ECs3725	ECs4242	ECs4870
ECs0075	ECs0664	ECs1481	ECs2470	ECs3187	ECs3737	ECs4243	ECs4871
ECs0076	ECs0665	ECs1482	ECs2471	ECs3188	ECs3738	ECs4244	ECs4872
ECs0077	ECs0666	ECs1483	ECs2472	ECs3189	ECs3739	ECs4245	ECs4873
ECs0078	ECs0667	ECs1484	ECs2474	ECs3190	ECs3740	ECs4246	ECs4874
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ECs0441	ECs1005	ECs2131	ECs2872	ECs3474	ECs4043	ECs4637	ECs5228
ECs0443	ECs1006	ECs2132	ECs2873	ECs3475	ECs4044	ECs4638	ECs5229
ECs0444	ECs1007	ECs2133	ECs2878	ECs3476	ECs4045	ECs4639	ECs5231
ECs0445	ECs1008	ECs2135	ECs2879	ECs3477	ECs4046	ECs4640	ECs5232
ECs0446	ECs1009	ECs2136	ECs2880	ECs3478	ECs4047	ECs4641	ECs5233
ECs0447	ECs1010	ECs2137	ECs2881	ECs3479	ECs4048	ECs4644	ECs5234
ECs0448	ECs1011	ECs2138	ECs2882	ECs3480	ECs4049	ECs4645	ECs5235
ECs0449	ECs1012	ECs2139	ECs2883	ECs3481	ECs4050	ECs4646	ECs5236
ECs0450	ECs1013	ECs2141	ECs2884	ECs3482	ECs4051	ECs4647	ECs5237
ECs0451	ECs1014	ECs2144	ECs2885	ECs3518	ECs4052	ECs4648	ECs5238
ECs0452	ECs1015	ECs2145	ECs2886	ECs3520	ECs4053	ECs4649	ECs5239
ECs0453	ECs1017	ECs2146	ECs2887	ECs3521	ECs4054	ECs4650	ECs5240
ECs0455	ECs1018	ECs2148	ECs2888	ECs3522	ECs4055	ECs4651	ECs5241
ECs0456	ECs1019	ECs2149	ECs2889	ECs3523	ECs4056	ECs4660	ECs5280
ECs0457	ECs1020	ECs2150	ECs2890	ECs3524	ECs4057	ECs4661	ECs5281
ECs0458	ECs1022	ECs2151	ECs2891	ECs3525	ECs4058	ECs4662	ECs5282
ECs0459	ECs1025	ECs2152	ECs2892	ECs3526	ECs4059	ECs4663	ECs5283
ECs0460	ECs1026	ECs2273	ECs2893	ECs3527	ECs4060	ECs4664	ECs5284
ECs0463	ECs1027	ECs2276	ECs2894	ECs3528	ECs4061	ECs4671	ECs5285
ECs0464	ECs1029	ECs2277	ECs2895	ECs3529	ECs4062	ECs4672	ECs5286
ECs0465	ECs1030	ECs2288	ECs2896	ECs3530	ECs4063	ECs4673	ECs5287
ECs0466	ECs1031	ECs2289	ECs2897	ECs3531	ECs4064	ECs4674	ECs5288
ECs0467	ECs1032	ECs2290	ECs2899	ECs3532	ECs4065	ECs4675	ECs5297
ECs0468	ECs1033	ECs2291	ECs2900	ECs3533	ECs4066	ECs4676	ECs5298
ECs0469	ECs1034	ECs2292	ECs2901	ECs3536	ECs4067	ECs4677	ECs5299
ECs0470	ECs1035	ECs2293	ECs2902	ECs3537	ECs4068	ECs4678	ECs5300
ECs0471	ECs1036	ECs2294	ECs2903	ECs3538	ECs4069	ECs4679	ECs5303
ECs0473	ECs1037	ECs2296	ECs2904	ECs3539	ECs4070	ECs4680	ECs5305
ECs0474	ECs1038	ECs2297	ECs2905	ECs3540	ECs4071	ECs4681	ECs5307
ECs0475	ECs1039	ECs2298	ECs2906	ECs3541	ECs4072	ECs4682	ECs5308
ECs0476	ECs1040	ECs2299	ECs2907	ECs3542	ECs4073	ECs4683	ECs5310
ECs0477	ECs1041	ECs2300	ECs2911	ECs3543	ECs4074	ECs4684	ECs5311
ECs0478	ECs1042	ECs2301	ECs2912	ECs3544	ECs4075	ECs4685	ECs5312
ECs0479	ECs1043	ECs2302	ECs2913	ECs3545	ECs4076	ECs4686	ECs5313
ECs0480	ECs1044	ECs2303	ECs2919	ECs3546	ECs4077	ECs4687	ECs5315
ECs0481	ECs1045	ECs2305	ECs2920	ECs3547	ECs4078	ECs4688	ECs5316

ECs0482	ECs1046	ECs2306	ECs2925	ECs3549	ECs4079	ECs4689	ECs5317
ECs0483	ECs1047	ECs2307	ECs2926	ECs3550	ECs4080	ECs4690	ECs5318
ECs0484	ECs1048	ECs2308	ECs2927	ECs3551	ECs4081	ECs4691	ECs5319
ECs0485	ECs1049	ECs2309	ECs2930	ECs3552	ECs4082	ECs4692	ECs5320
ECs0486	ECs1050	ECs2310	ECs2934	ECs3553	ECs4083	ECs4693	ECs5321
ECs0487	ECs1051	ECs2311	ECs2935	ECs3554	ECs4084	ECs4694	ECs5322
ECs0488	ECs1052	ECs2312	ECs2936	ECs3555	ECs4085	ECs4695	ECs5323
ECs0489	ECs1053	ECs2313	ECs2937	ECs3556	ECs4086	ECs4696	ECs5324
ECs0490	ECs1054	ECs2314	ECs3014	ECs3557	ECs4087	ECs4697	ECs5325
ECs0491	ECs1094	ECs2315	ECs3015	ECs3558	ECs4088	ECs4698	ECs5326
ECs0492	ECs1128	ECs2316	ECs3016	ECs3560	ECs4089	ECs4699	ECs5327
ECs0493	ECs1129	ECs2317	ECs3017	ECs3561	ECs4090	ECs4700	ECs5328
ECs0494	ECs1130	ECs2318	ECs3018	ECs3562	ECs4091	ECs4701	ECs5329
ECs0495	ECs1131	ECs2319	ECs3019	ECs3563	ECs4092	ECs4702	ECs5330
ECs0496	ECs1132	ECs2320	ECs3020	ECs3564	ECs4094	ECs4703	ECs5331
ECs0497	ECs1133	ECs2323	ECs3021	ECs3565	ECs4095	ECs4704	ECs5332
ECs0498	ECs1134	ECs2326	ECs3022	ECs3567	ECs4096	ECs4705	ECs5333
ECs0499	ECs1135	ECs2327	ECs3023	ECs3568	ECs4097	ECs4706	ECs5334
ECs0500	ECs1136	ECs2328	ECs3024	ECs3569	ECs4098	ECs4707	ECs5335
ECs0501	ECs1137	ECs2329	ECs3026	ECs3570	ECs4099	ECs4708	ECs5336
ECs0502	ECs1138	ECs2330	ECs3033	ECs3571	ECs4101	ECs4709	ECs5337
ECs0503	ECs1139	ECs2331	ECs3034	ECs3572	ECs4102	ECs4710	ECs5340
ECs0504	ECs1140	ECs2332	ECs3035	ECs3573	ECs4103	ECs4711	ECs5341
ECs0505	ECs1141	ECs2333	ECs3036	ECs3574	ECs4104	ECs4712	ECs5342
ECs0506	ECs1142	ECs2334	ECs3037	ECs3575	ECs4105	ECs4713	ECs5343
ECs0507	ECs1143	ECs2335	ECs3038	ECs3576	ECs4106	ECs4714	ECs5344
ECs0508	ECs1144	ECs2336	ECs3039	ECs3577	ECs4107	ECs4715	ECs5345
ECs0509	ECs1145	ECs2337	ECs3040	ECs3578	ECs4108	ECs4716	ECs5346
ECs0510	ECs1146	ECs2339	ECs3041	ECs3579	ECs4109	ECs4717	ECs5347
ECs0511	ECs1147	ECs2340	ECs3042	ECs3580	ECs4110	ECs4718	ECs5348
ECs0512	ECs1148	ECs2341	ECs3043	ECs3581	ECs4111	ECs4719	ECs5349
ECs0513	ECs1149	ECs2342	ECs3044	ECs3582	ECs4112	ECs4720	ECs5350
ECs0514	ECs1150	ECs2343	ECs3045	ECs3583	ECs4113	ECs4721	ECs5351
ECs0515	ECs1151	ECs2344	ECs3046	ECs3584	ECs4114	ECs4722	ECs5352
ECs0516	ECs1152	ECs2345	ECs3047	ECs3585	ECs4115	ECs4723	ECs5353
ECs0517	ECs1153	ECs2346	ECs3048	ECs3586	ECs4116	ECs4724	ECs5354
ECs0518	ECs1154	ECs2347	ECs3049	ECs3587	ECs4117	ECs4725	ECs5355
ECs0520	ECs1155	ECs2348	ECs3050	ECs3588	ECs4118	ECs4726	ECs5356
ECs0521	ECs1158	ECs2349	ECs3051	ECs3589	ECs4119	ECs4727	ECs5357
ECs0522	ECs1159	ECs2350	ECs3053	ECs3590	ECs4120	ECs4728	ECs5358
ECs0523	ECs1250	ECs2351	ECs3054	ECs3591	ECs4121	ECs4729	ECs5359
ECs0524	ECs1252	ECs2352	ECs3055	ECs3592	ECs4122	ECs4730	ECs5360
ECs0525	ECs1253	ECs2353	ECs3056	ECs3593	ECs4123	ECs4732	ECs5361
ECs0526	ECs1254	ECs2354	ECs3057	ECs3594	ECs4124	ECs4734	ECs5362
ECs0527	ECs1255	ECs2355	ECs3058	ECs3595	ECs4125	ECs4735	ECs5363
ECs0528	ECs1256	ECs2356	ECs3059	ECs3596	ECs4127	ECs4736	ECs5364
ECs0529	ECs1257	ECs2357	ECs3060	ECs3597	ECs4128	ECs4738	ECs5365
ECs0530	ECs1258	ECs2358	ECs3061	ECs3598	ECs4129	ECs4739	ECs5375
ECs0531	ECs1259	ECs2359	ECs3062	ECs3599	ECs4130	ECs4740	ECs5377
ECs0532	ECs1260	ECs2360	ECs3063	ECs3600	ECs4131	ECs4741	ECs5380

ECs0533	ECs1261	ECs2361	ECs3064	ECs3601	ECs4132	ECs4742	ECs5381
ECs0534	ECs1263	ECs2362	ECs3065	ECs3602	ECs4133	ECs4743	ECs5382
ECs0535	ECs1264	ECs2363	ECs3066	ECs3603	ECs4134	ECs4745	ECs5383
ECs0536	ECs1265	ECs2364	ECs3067	ECs3604	ECs4135	ECs4747	ECs5391
ECs0537	ECs1267	ECs2365	ECs3068	ECs3605	ECs4136	ECs4748	ECs5411
ECs0538	ECs1268	ECs2366	ECs3069	ECs3606	ECs4137	ECs4749	ECs5414
ECs0539	ECs1269	ECs2367	ECs3070	ECs3607	ECs4140	ECs4750	ECs5421
ECs0545	ECs1270	ECs2368	ECs3071	ECs3608	ECs4141	ECs4751	ECs5423
ECs0552	ECs1271	ECs2369	ECs3072	ECs3609	ECs4142	ECs4752	ECs5426
ECs0553	ECs1297	ECs2370	ECs3073	ECs3610	ECs4143	ECs4753	ECs5432
ECs0554	ECs1298	ECs2371	ECs3074	ECs3611	ECs4144	ECs4754	ECs5460
ECs0555	ECs1299	ECs2372	ECs3075	ECs3612	ECs4145	ECs4755	ECs5463
ECs0556	ECs1304	ECs2373	ECs3076	ECs3613	ECs4146	ECs4756	ECs5464
ECs0557	ECs1343	ECs2374	ECs3077	ECs3614	ECs4147	ECs4757	ECs5484
ECs0558	ECs1344	ECs2375	ECs3078	ECs3615	ECs4148	ECs4758	ECs5486
ECs0559	ECs1345	ECs2376	ECs3079	ECs3616	ECs4149	ECs4759	ECs5487
ECs0564	ECs1346	ECs2377	ECs3080	ECs3617	ECs4150	ECs4760	ECs5495
ECs0565	ECs1348	ECs2378	ECs3081	ECs3618	ECs4151	ECs4761	ECs5496
ECs0566	ECs1349	ECs2379	ECs3082	ECs3619	ECs4152	ECs4762	ECs5497
ECs0567	ECs1350	ECs2380	ECs3083	ECs3620	ECs4153	ECs4763	ECs5498
ECs0568	ECs1351	ECs2381	ECs3084	ECs3621	ECs4154	ECs4764	ECs5530
ECs0569	ECs1352	ECs2382	ECs3085	ECs3622	ECs4155	ECs4765	ECs5531
ECs0570	ECs1353	ECs2383	ECs3086	ECs3623	ECs4156	ECs4766	ECs5532
ECs0571	ECs1354	ECs2384	ECs3087	ECs3626	ECs4157	ECs4767	ECs5533
ECs0572	ECs1358	ECs2385	ECs3088	ECs3629	ECs4158	ECs4768	ECs5536
ECs0575	ECs1359	ECs2386	ECs3089	ECs3630	ECs4159	ECs4769	ECs5537
ECs0576	ECs1360	ECs2387	ECs3090	ECs3631	ECs4160	ECs4770	ECs5541
ECs0577	ECs1362	ECs2388	ECs3091	ECs3632	ECs4161	ECs4771	ECs5549
ECs0578	ECs1364	ECs2389	ECs3092	ECs3633	ECs4162	ECs4772	ECs5556
ECs0579	ECs1365	ECs2390	ECs3093	ECs3639	ECs4163	ECs4773	ECs5562
ECs0580	ECs1366	ECs2391	ECs3094	ECs3640	ECs4164	ECs4774	ECs5575
ECs0581	ECs1367	ECs2392	ECs3096	ECs3641	ECs4165	ECs4775	ECs5584
ECs0582	ECs1370	ECs2393	ECs3097	ECs3642	ECs4166	ECs4776	ECs5586
ECs0583	ECs1374	ECs2394	ECs3098	ECs3643	ECs4167	ECs4777	ECs5593
ECs0584	ECs1375	ECs2395	ECs3099	ECs3644	ECs4168	ECs4778	ECs5596
ECs0585	ECs1376	ECs2396	ECs3100	ECs3645	ECs4169	ECs4779	
ECs0586	ECs1384	ECs2397	ECs3101	ECs3646	ECs4170	ECs4780	
ECs0587	ECs1386	ECs2398	ECs3102	ECs3647	ECs4171	ECs4781	
ECs0588	ECs1388	ECs2399	ECs3103	ECs3648	ECs4172	ECs4782	
ECs0589	ECs1389	ECs2400	ECs3104	ECs3649	ECs4173	ECs4783	
ECs0590	ECs1409	ECs2401	ECs3105	ECs3650	ECs4174	ECs4784	
ECs0591	ECs1410	ECs2402	ECs3106	ECs3651	ECs4175	ECs4785	
ECs0592	ECs1411	ECs2403	ECs3107	ECs3652	ECs4176	ECs4786	
ECs0593	ECs1413	ECs2404	ECs3108	ECs3653	ECs4177	ECs4787	
ECs0594	ECs1414	ECs2405	ECs3109	ECs3654	ECs4178	ECs4788	
ECs0595	ECs1415	ECs2406	ECs3110	ECs3655	ECs4179	ECs4789	

Supplementary Video 1. Time-lapse observation of HCT-8 cells exposed to LB226692 OMVs. (**upper panels**) Videos of stacked white light images (taken at 30 min intervals) of HCT-8 cells incubated with cell culture medium (control; left) or OMVs LB226692 (containing 580 ng/ml of Stx2a) (right). (**lower panels**) Videos of stacked segmented (green outlines) quantitative DHM phase contrast images of the corresponding measurements in the upper panels. The scale bar is valid for all videos and corresponds to 20 μm (lower left panel). The corresponding time of the shown frames is depicted in the upper left panel.