Electronic Supplementary Material (ESI) for Soft Matter.

SUPPLEMENTARY MATERIAL

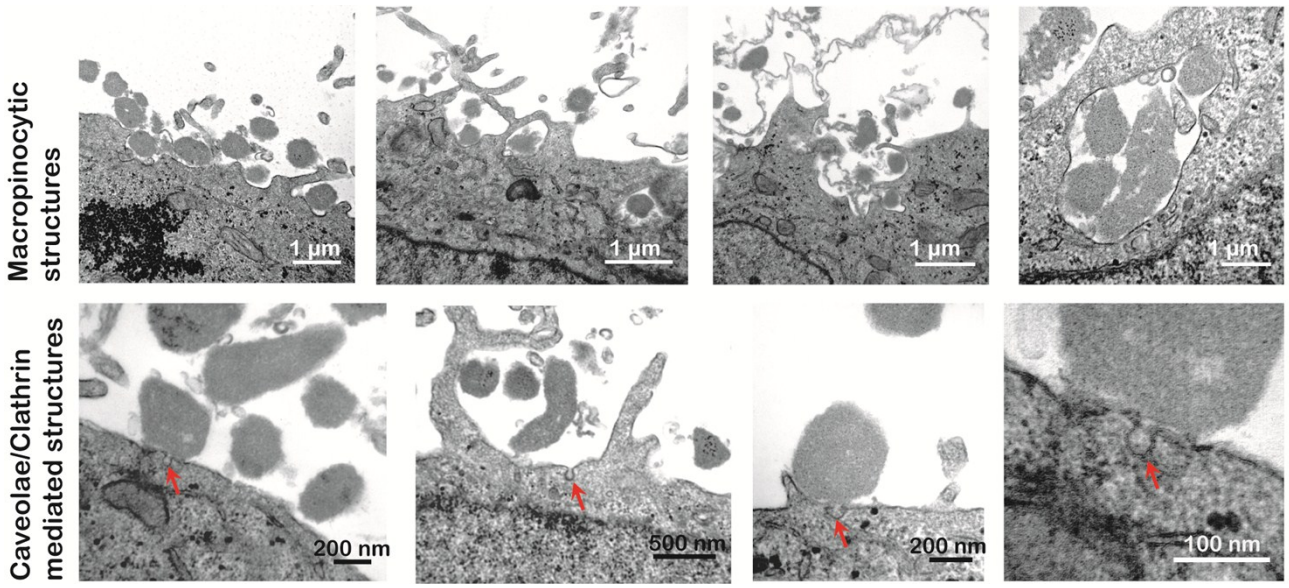

Figure 1 SI. Panels above, TEM micrographs showing different morphological structures characteristic of macropinocytosis endocytic pathway (membrane ruffling, non-homogeneous in size vesicles and with irregular shapes). Panels below, TEM images showing classical invaginations of caveolae and clathrin mediated endocytosis, indicated by red arrows.

1. *Field Emission Scanning Electron Microscopy (FESEM)*

For IBs ultrastructure, a set of IB samples were filtered and processed as previously described and observed without coating in a FESEM Zeiss Merlin operating of 2 kV. Images of IBs morphology were acquired with a high resolution in-*lens secondary electron* (SE) detector. A drop of another set of IB samples were deposited on a silicon surface, air dried and observed in the FESEM Zeiss Merlin under the same conditions.

For GFP Immunolocalization in HeLA-samples, coverslips of HeLa cultures incubated with IBs were fixed in 4 % paraformaldehyde (TAAB Lab., UK) and 0.1 % (v/v) glutaraldehyde in 0.1 M PB for 2 h at 4 ºC. Samples were blocked 30 min in 1 % (w/v) bovine serum albumin (BSA; Sigma-Aldrich, Germany) in PBS (BSA/PBS) containing 20 mM of glycine (Sigma-Aldrich, Germany), incubated with the primary polyclonal antibody anti-GFP (Santa Cruz, USA) at dilution 1:50 in 1 % (w/v) BSA/PBS at 4 ºC, washed in 1 % BSA/PBS and incubated in protein A coupled to 10 nm-gold particles (CMC Utrecht, NL) in 1 % BSA/PBS for 40 min, washed firstly in PBS and followed by 5 washes with deionized water. Samples were then dehydrated in ascending ethanol series and dried with CO_2 as reported previously for ultrastructural studies with SEM. Samples were observed without coating in a FESEM Zeiss Merlin operating of 2 kV. Images of morphology were acquired with a high resolution in-*lens secondary electron* (SE) detector and gold particles of labeling were localized with an energy selective back-scattered electron (ESB) detector.

For GFP fluorescence of IBs, ultrathin sections of Lowicryl HM20 embedded samples of IBs and HeLa incubated with IBs (detailed protocol in TEM GFP and DnaK immunolocalization in HeLa-IBs subsection) were placed in carbon coated copper grips and observed in a FESEM Zeiss Merlin operating of 2 kV and fluorescence was imagined with an energy selective back-scattered electron (ESB) detector.

2. *Transmission Electron Microscopy (TEM)*

For IBs negative staining, a drop of 3-5 µl of IBs sample was deposited during 2 min on 200 mesh copper grids coated with carbon, contrasted with uranyl acetate 2% during 2 min, air dried and observed with a transmission electron microscope Jeol JEM-1400 (Jeol Ltd., Japan) equipped with a CCD Gatan ES1000W Erlangshen camera.

For GFP immunolocalization in IBs, a drop of 3-5 µl of IBs sample was deposited during 2 min in 200 mesh copper grids coated with carbon, fixed with 4 % (w/v) paraformaldehyde and 0.1 % (v/v) glutaraldehyde in 0.1 M PB during 5 min, washed and labelled as previously described for FESEM. Samples were then contrasted with uranyl acetate 2 % during 2 min, air dried and observed with a TEM Jeol JEM-1400.

For ultrastructure of HeLa-IBs interaction, pellets of HeLa incubated with IBs at different times (0, 0.5, 3, 8 and 24 h) and control samples of HeLa cells were fixed overnight in 2 % (w/v) paraformaldehyde and 2.5 % (v/v) glutaraldehyde in 0.1 M PB, post-fixed for 2 h with 1 % (w/v) osmium tetroxide containing 0.8 % (w/v) potassium hexocyanoferrate in PB, sequentially dehydrated in acetone, embedded in Eponate 12™ resin (Ted Pella, Inc, USA), and polymerized at 60 °C for 48 h. Ultrathin sections (70 nm) of selected areas of semithin sections (1 µm) were obtained with Leica ultracut UCT microtome (Leica Microsystems, Germany), placed on 200 mesh copper grids, and contrasted with conventional uranyl acetate (30 min) and lead citrate (5 min) solutions as detailed elsewhere [1,2] Grids were observed with a TEM Jeol JEM-1400.

For GFP and DnaK immunolocalization in HeLa-IBs, cell pellets of HeLa incubated with IBs at different times were fixed in 4 % (w/v) paraformaldehyde and 0.1 % (v/v) glutaraldehyde in 0.1 M PB for 30 min and stored in 1% (w/v) paraformaldehyde prepared in PB. Samples were placed with 20 mM glycine, cryoprotected in a graded series of sucrose (0.7, 1.4 and 2.3 M; Sigma-Aldrich, Germany) solutions prepared in PB, and cryofixed in liquid propane in an EMCPC (Leica Microsystems, Germany). Samples were immersed in methanol (Merck, Germany) containing 0.5 uranyl acetate for 72 h at -90 ºC, washed with methanol, embedded in Lowicryl HM20 resin (Polysciences Inc., USA), and polymerized with UV rays for 48 h at -45

°C followed by 48 h at 25 ºC in a EMAFS automatic freeze substitution system (Leica Microsystems, Germany). Ultrathin 80 nm sections placed on 200 mesh gold grids without coating were labelled for GFP as previously described for SEM, contrasted, and examined with a TEM Jeol JEM-1400. Double labelling of GFP and DnaK was performed in other set of Lowicryl HM20 embedded sections placed in gold grids without coating and potential cross-reactivity was avoided by applying each primary and subsequent secondary antibody onto a different face of the grid.[3,4] DnaK labelling was carried out with the same procedure than the described for GFP samples but using a rabbit anti-DnaK serum as primary antibody and protein A coupled to 20 nm-gold particles. Grids were contrasted with uranyl acetate (15 min) and lead citrate (1 min) and observed with a TEM Jeol JEM-1400.

Reference List

[1] Rodriguez-Carino, C., Duffy, C., Sanchez-Chardi, A., McNeilly, F., Allan, G. M., and Segales, J. Porcine circovirus type 2 morphogenesis in a clone derived from the l35 lymphoblastoid cell line, J.Comp Pathol. 144 (2011) 91-102

[2] Mussa, T., Rodriguez-Carino, C., Sanchez-Chardi, A., Baratelli, M., Costa-Hurtado, M., Fraile, L., Dominguez, J., Aragon, V., and Montoya, M. Differential interactions of virulent and non-virulent H. parasuis strains with naive or swine influenza virus pre-infected dendritic cells, Vet.Res. 43 (2012) 80

[3] Bendayan, M. Double immunocytochemical labeling applying the protein A-gold technique, J.Histochem.Cytochem. 30 (1982) 81-85

[4] Rodriguez-Carino, C., Sanchez-Chardi, A., and Segales, J. Subcellular immunolocalization of porcine circovirus type 2 (PCV2) in lymph nodes from pigs with post-weaning multisystemic wasting syndrome (PMWS), J.Comp Pathol. 142 (2010) 291-299