

## Extracellular vesicles in arbovirus infections: from basic biology to potential clinical applications

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### Supplementary Material

**Supplementary Table S1. Summary of the most relevant observations regarding EVs released by *in vitro* infected cells and their functional properties on target cells**

| Infected cell type                  | Pathogen strain                  | EV enrichment method            | Properties of EVs from infected cells                                                          | Cargo of viral material      | Effects on naive cells                                                                   | Controls in EV preparation                      | Ref. |
|-------------------------------------|----------------------------------|---------------------------------|------------------------------------------------------------------------------------------------|------------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------|------|
| <b>DENV</b>                         |                                  |                                 |                                                                                                |                              |                                                                                          |                                                 |      |
| Platelets                           | DENV-2 (16881)                   | Low speed centrifugation        | Increased release of EVs and IL-1 $\beta$ <sup>+</sup> EVs                                     | nd                           | Increased endothelial (HMEC-1) permeability                                              | nd                                              | (1)  |
| Platelets (DENV patients)           | na                               | UC                              | Increased secretion of EVs                                                                     | nd                           | Loss of adhesion molecules and increased vascular inflammation in HUVECs                 | nd                                              | (2)  |
| Platelets                           | DENV2 (PL046 and New Guinea C-N) | Low speed centrifugation + UC   | Upregulated CD62p <sup>+</sup> /CD41 <sup>+</sup> and CD63 <sup>+</sup> /CD41 <sup>+</sup> EVs | nd                           | Induction of NET formation in neutrophils; Increased vascular permeability in HMEC-1     | nd                                              | (3)  |
| HepG2 HUVECs                        | DENV2 (16881)                    | Differential centrifugation     | Increased release of AnV <sup>+</sup> EVs                                                      | E and NS1 on the surface     | nd                                                                                       | nd                                              | (4)  |
| THP1 HEK293T (DENV-NS1-transfected) | DENV2 (New Guinea C)             | Salting out                     | Increased release of EVs enriched with miR-148a                                                | nd                           | Enhancement of neuro-inflammation in microglial cells (CHEM3)                            | EVs from HEK293T transfected with DENV-NS1 gene | (5)  |
| Monocyte-derived DCs                | DENV3 (5532 and 290)             | UC                              | Altered miRNA and mRNA cargo                                                                   | Viral RNA or viral particles | Infective in C6/36 cells                                                                 | 4G2-Anti-dengue E protein antibody              | (6)  |
| U937                                | DENV2 (Colombian isolated)       | UC + sucrose gradient + CD36 IP | Heterogeneous EV population, protein cargo involved in                                         | NS3 protein                  | Endothelial activation (EA.hy926)<br>Non-infective in epithelial cells (LLC-MK2, monkey) | UV irradiation                                  | (7)  |

|                                                                     |                                                         |                                                         |                                                            |                                                    |                                                                                   |                                                             |      |
|---------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------|------|
|                                                                     |                                                         |                                                         | the immune response                                        |                                                    |                                                                                   |                                                             |      |
| C6/36<br>( <i>A. albopictus</i> )<br>Aag-2<br>( <i>A. aegypti</i> ) | DENV2 (TVP2176, New Guinea C)<br>DENV3 (BID-V1619/2005) | OptiPrep DG or exosome isolation kit or UC              | EVs size range of 30-250 nm; increased amount of small EVs | Viral RNA (full-length genome), E and NS1 proteins | Infective in C6/36 cells, Vero E6, mouse DCs, human-skin keratinocytes and HUVECs | RNase A and Triton X-100<br>4G2-antibody bead binding assay | (8)  |
| C6/36<br>( <i>A. albopictus</i> )                                   | DENV2 (New Guinea C)                                    | UC + exosome isolation kit + CD9 <sup>+</sup> selection | Larger size EVs                                            | Virus-like particles                               | Infective in C6/36                                                                | Immuno-magnetic bead affinity purification (CD9)            | (9)  |
| ATC-10<br>( <i>A. aegypti</i> )                                     | DENV2 (New Guinea C)                                    | Exosome isolation kit                                   | Altered protein cargo, loaded with AAEL00275 protein       | nd                                                 | Infection enhancing capacity of AAEL00275 in human primary dermal fibroblasts     | nd                                                          | (10) |
| <i>A. albopictus</i> , saliva                                       | DENV2 (New Guinea C)                                    | None                                                    | EV-like particles of 100-500 nm                            | 3'UTR sequences, Subgenomic RNA                    | nd                                                                                | Triton X-100, RNase A and Proteinase K                      | (11) |
| <b>ZIKV</b>                                                         |                                                         |                                                         |                                                            |                                                    |                                                                                   |                                                             |      |
| Primary astrocytes                                                  | ZIKV MR766 and PRVABC59                                 | UC                                                      | Increased release of EVs                                   | nd                                                 | nd                                                                                | nd                                                          | (12) |
| Primary cortical neurons (mouse)                                    | ZIKV PRVABC59                                           | UC or DG                                                | Increased release of EVs                                   | RNA, E protein                                     | Infective in neuronal cells                                                       | RNase A, neutralizing antibodies                            | (13) |
| SNB-19                                                              | ZIKV PRVABC59                                           | UC + gradient purification                              | Increased release of EV with wide size range and density   | RNA, E protein                                     | Infective in Vero E6                                                              | RNase A and Triton X-100 treatment                          | (14) |
| hcMEC/D3                                                            | ZIKV PRVABC59                                           | Differential centrifugation + ultra-filtration + DG     | Altered lipid cargo                                        | RNA, E and NS1 proteins                            | Transfer of viral material to hcMEC/D3 and glioblastoma cells                     | RNase A treatment                                           | (15) |
| Early gestation trophoblasts (TSCs, macaque)                        | ZIKV DAK AR 41524 NR-50338 ("DAKAR")                    | Size exclusion chromatography                           | Altered mRNA, miRNA and protein cargo                      | nd                                                 | nd                                                                                | nd                                                          | (16) |

|                                 |                                       |                                                  |                                                                          |                                                  |                                                                                                                                                    |                                        |      |
|---------------------------------|---------------------------------------|--------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|------|
| JEG-3 cells                     | ZIKV MR766 and PRVABC59               | Exo-Spin kit                                     | Altered cargo of miRNA and proteins                                      | NS1 protein                                      | nd                                                                                                                                                 | nd                                     | (17) |
| THP1                            | ZIKV MR766                            | UC                                               | na                                                                       | E and NS1 proteins, virus like particles         | Infective in Vero E6 and monocytes. Monocyte activation and differentiation                                                                        | UV irradiation, RNase A                | (18) |
| C6/36 ( <i>A. albopictus</i> )  | ZIKV MR766                            | UC                                               | Release of medium (AnV <sup>+</sup> ) and small (CD63 <sup>+</sup> ) EVs | Viral RNA and E protein                          | Infective in Vero E6, THP-1, HMEC-1. Promotion of monocyte differentiation and activation; alteration of endothelial permeability and inflammation | UV irradiation, RNase A                | (18) |
| HUVECs                          | Stabilized infectious cDNA clone pZL1 | UC + CD9 immuno-capture                          | E protein on EVs surface and binding to neutralizing antibodies          | Capsid, prM, E, NS1, and NS5 proteins, viral RNA | Non-infective in Vero-E6                                                                                                                           | EV affinity enrichment                 | (19) |
| <b>WNV</b>                      |                                       |                                                  |                                                                          |                                                  |                                                                                                                                                    |                                        |      |
| A549                            | WNV Kunjin                            | ExoQuick-TC                                      | Altered miRNAs, RNAs, ncRNAs cargo                                       | nd                                               | nd                                                                                                                                                 | Treatment with heat (56°C) and RNase A | (20) |
| N2a (mouse)                     | WNV CT2741 wild-type                  | OptiPrep DG                                      | na                                                                       | RNA                                              | Infective in N2a                                                                                                                                   | nd                                     | (21) |
| <b>JEV</b>                      |                                       |                                                  |                                                                          |                                                  |                                                                                                                                                    |                                        |      |
| N9 Microglial cells (mouse)     | JEV GP78                              | Exosome isolation reagent                        | Increased let-7a and let-7b miRNAs                                       | nd                                               | Accelerate neuronal damage in N2a cells                                                                                                            | nd                                     | (22) |
| <b>CHIKV</b>                    |                                       |                                                  |                                                                          |                                                  |                                                                                                                                                    |                                        |      |
| Vero E6 (green monkey)          | CHIKV ROSS C6D4                       | Total exosome isolation kit + CD63 isolation kit | Release of small EVs                                                     | E1 mRNA, Viral genome                            | Infective in Vero E6 cells                                                                                                                         | RNase A and Triton X-100               | (23) |
| <b>LGTV (as model for TBEV)</b> |                                       |                                                  |                                                                          |                                                  |                                                                                                                                                    |                                        |      |
| ISE6 ( <i>I. scapularis</i> )   | LGTV LGT-TP21                         | OptiPrep DG                                      | Size range: 30-200nm                                                     | E and NS1 proteins, viral RNA                    | Infective in human keratinocytes and HUVEC                                                                                                         | Triton X-100, 4G2 antibody             | (21) |
| bEnd.3 (mouse)                  | LGTV LGT-TP21                         | OptiPrep DG                                      | Size range: 30-200nm                                                     | Viral RNA                                        | Infective in N2a cells                                                                                                                             | nd                                     | (21) |

|                   |               |              |                      |                      |                         |                          |      |
|-------------------|---------------|--------------|----------------------|----------------------|-------------------------|--------------------------|------|
| N2a (mouse)       | LGTV LGT-TP21 | OptiPrep DG  | Size range: 30-200nm | E protein, viral RNA | Infective in N2a cells  | Proteinase K and RNase A | (21) |
| <b>SFTS virus</b> |               |              |                      |                      |                         |                          |      |
| HeLa              | SFTS virus    | UC + CD63 IP | na                   | NSs protein, virions | Infective in HeLa cells | IP, negative selection   | (24) |

*HepG2: liver epithelial cells; HUVECs: primary umbilical vein endothelial cells; THP1: monocyte cell line; HEK293T: embryonic kidney epithelial cell line; DCs: dendritic cells; hcMEC/D3: human brain microvascular endothelial cells; U937: macrophages; SNB-19: glioblastoma cell line; JEG-3: trophoblasts; A549: lung epithelial cells; N2a: murine neuroblastoma cell line; Vero E6: green monkey kidney epithelial cells; HeLa: cervical epithelial cells; bEnd.3: murine brain-microvascular endothelial cells. All cells are of human origin, unless otherwise specified.*

*UC: ultracentrifugation; DG: density gradient; IP: immunoprecipitation; na: not available; nd: not determined.*

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