# Cytochalasin B-induced membrane vesicles convey angiogenic activity of parental cells

## SUPPLEMENTARY MATERIALS

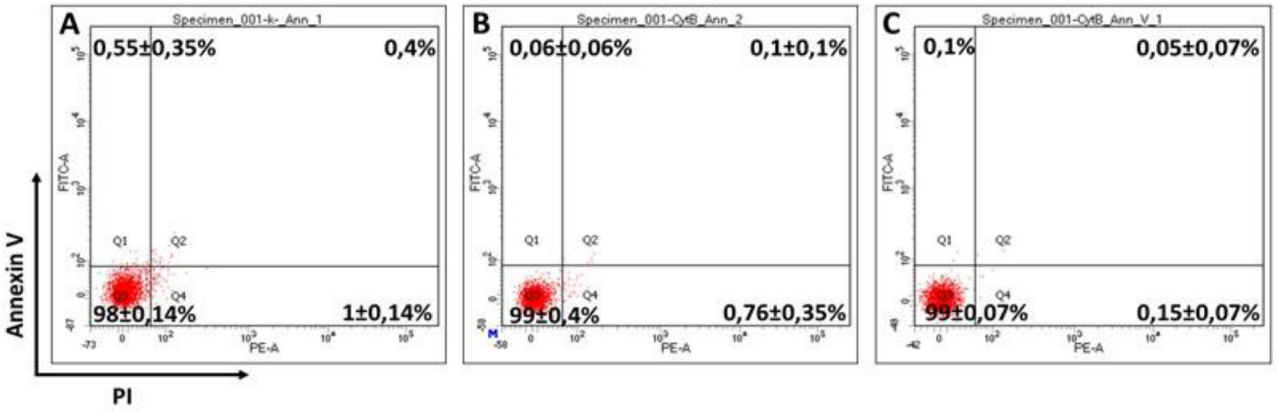
## RESULTS

### Influence of CIMVs method production on donor cell viability

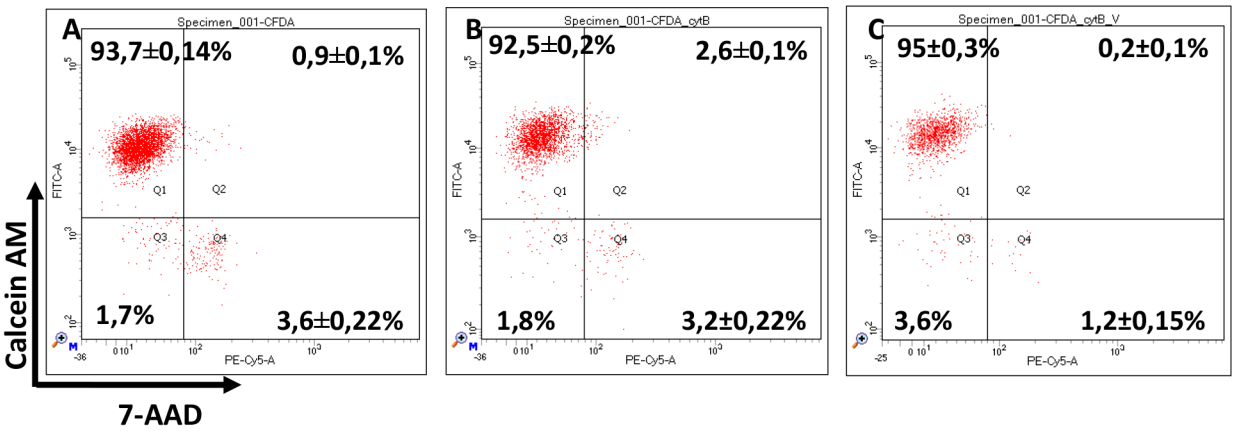
We performed cell viability tests by Annexin V/PI and Calcein AM/7-AAD staining to evaluate (I) SH-SY5Y cells viability after 30 min of Cytochalasin B treatment and (II) SH-SY5Y cells viability after 30 sec of vortexing. Staining with Annexin V/ PI and Calcein AM/ 7-AAD indicates cell viability is not impaired following 30 min of Cytochalasin B treatment and after subsequent 30 sec of vortexing (Supplementary Figure 1, 2).

### Influence of CIMVs on recipient cell viability

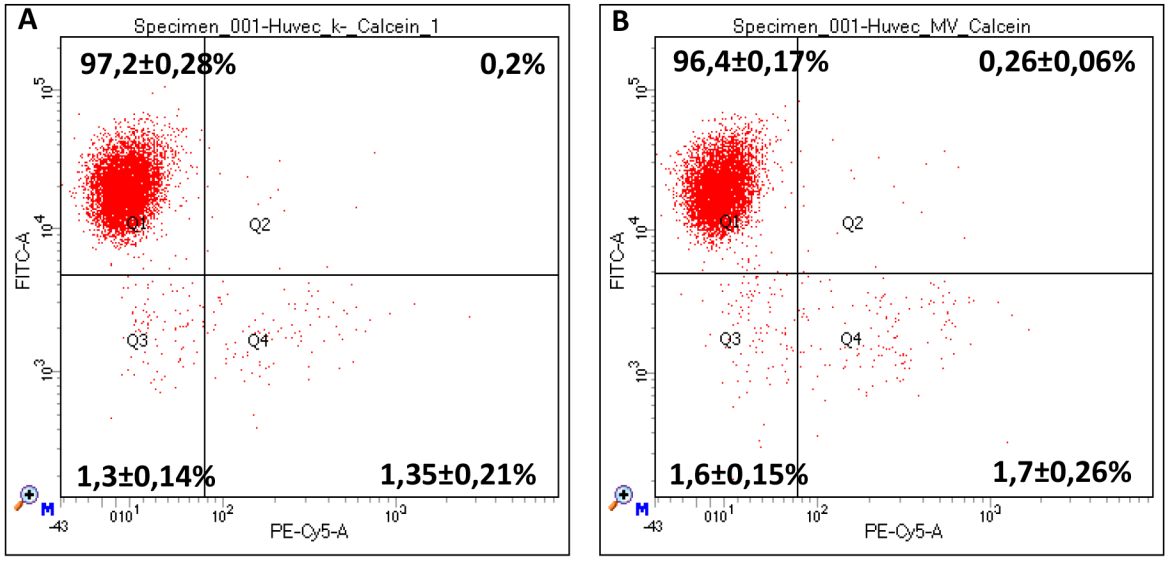
As cytochalasin B binds with actin filaments, trace amounts of cytochalasin B may be retained within CIMVs associated with actin fragments. We therefore evaluated HUVEC viability after addition of SH-SY5Y derived CIMVs using Calcein AM/ 7-AAD staining. SH-SY5Y derived CIMVs were added to the HUVEC culture ($2x10^4$ cells) at a concentration 60 μg/ml (Supplementary Figure 3).




**Supplementary Figure 1:** Annexin V/ PI staining of: **(A)** - control SH-SY5Y cells; **(B)** - SH-SY5Y cells after 30 min of Cytochalasin B treatment; **(C)** - SH-SY5Y cells after 30 min of Cytochalasin B treatment and subsequent 30 sec of vortexing.




**Supplementary Figure 2:** Calcein AM/ 7-AAD staining of: **(A)** - control SH-SY5Y cells; **(B)** - SH-SY5Y cells after 30 min of Cytochalasin B treatment; **(C)** - SH-SY5Y cells after 30 min of Cytochalasin B treatment and subsequent 30 sec of vortexing**.**




**Supplementary Figure 3:** Calcein AM/ 7-AAD staining of: **(A)** - control HUVEC; **(B)** - HUVEC after 16 hours of CIMVs SHSY5Y addition**.**