

Supplementary Materials:

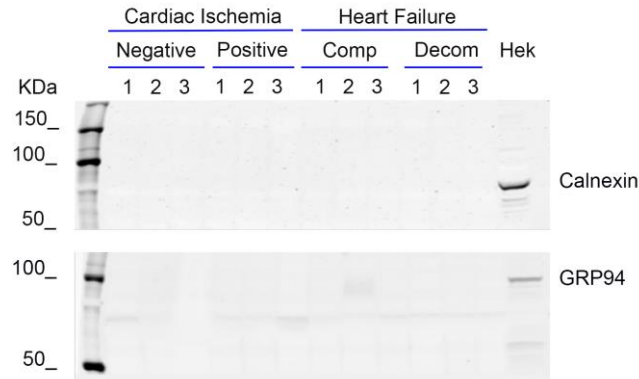


Figure S1. Calnexin and Grp94 were absent in the sEVs purified by ultracentrifugation. The presence of calnexin and Grp94 (MISEV2023. Protein content-based EV characterization as a negative control for sEVs [59]) was determined using Western blot, using a specific antibody for calnexin or Grp94. An equivalent amount of 1×10^9 sEVs was considered for the analysis. Three samples were considered for each group of participants with different diagnoses of CVS. Comp: compensated; Decom: decompensated; Hek: homogenized from HEK293 cells growing under normal condition.

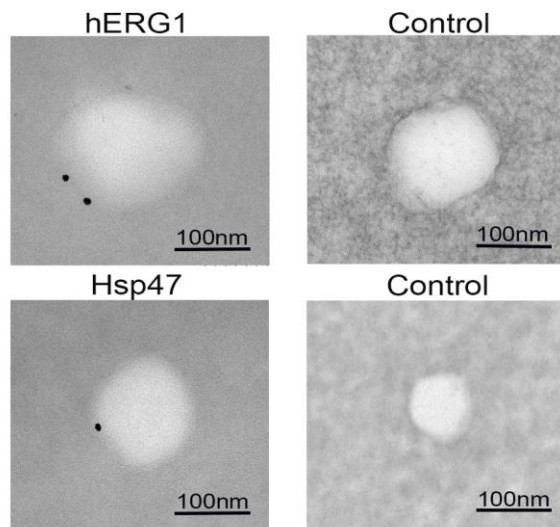


Figure S2. Transmission electron microscopy: sEVs purified from blood plasma by ultracentrifugation were incubated with anti-hERG1 or anti-Hsp47 antibodies. Then, they were incubated with a gold-conjugated secondary antibody (IgG-Au). The control consisted of sEVs incubated with only IgG-Au.

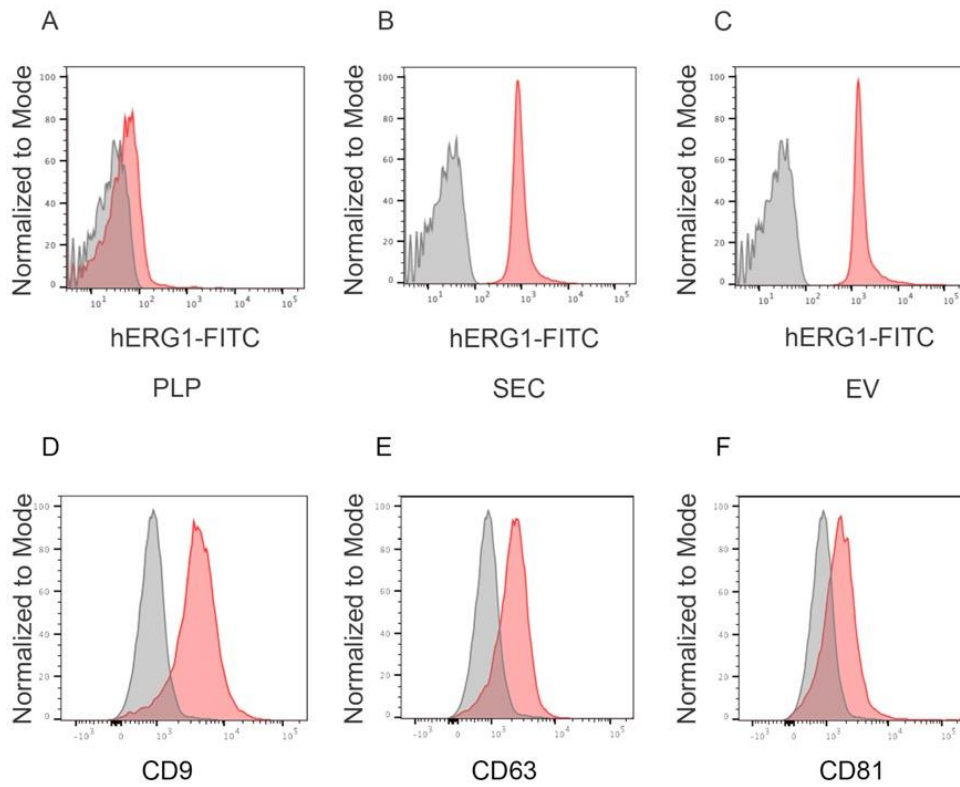
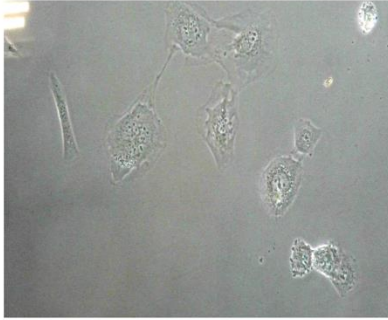


Figure S3. The expression of EV-hERG1 was detected using flow cytometry in blood plasma using one-step size exclusion chromatography sEVs purification. The presence of sEVs containing hERG1 (EV-hERG1) was determined using flow cytometry, using a specific antibody for hERG1 conjugated by FITC. An equivalent amount of 3.5×10^8 sEVs was considered for the analysis. Representative flow cytometry histograms of the presence of hERG1 using (A) platelet-free plasma (PLP); (B) size exclusion chromatography (SEC) sEVs purification; (C) extracellular vesicles (sEVs) purified using ultracentrifugation; (D) CD9 for extracellular vesicles (sEVs) purified using SEC; (E) CD63 for extracellular vesicles (sEVs) purified using SEC; (F) CD81 for extracellular vesicles (sEVs) purified using SEC. The grey region represents the basal signal, and the red region represents the hERG1 signal.

Cardiomyocyte cell line (AC-16)

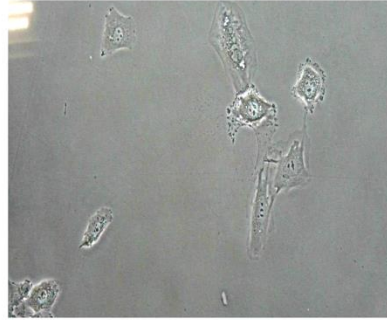
Normoxia

21 % oxygen
FBS-Free
1 hour

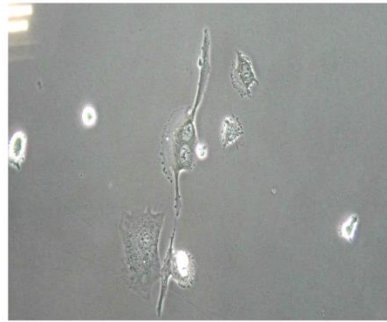
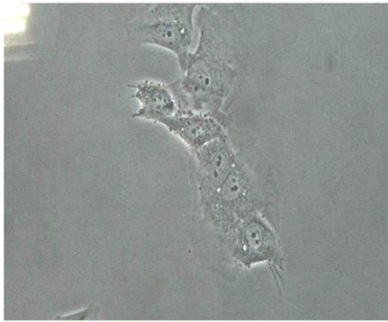


Hypoxia

1 % oxygen
FBS-Free
1 hour



20X



40X

Figure S4. human cardiomyocyte cell line (AC-16) exposed to normoxia (21% oxygen) and hypoxia (1% oxygen) in an FBS-free culture medium for 1 hour.