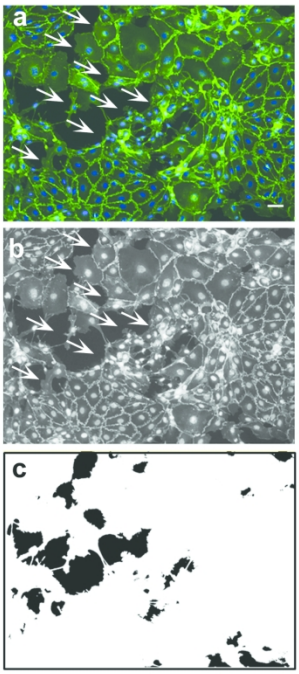
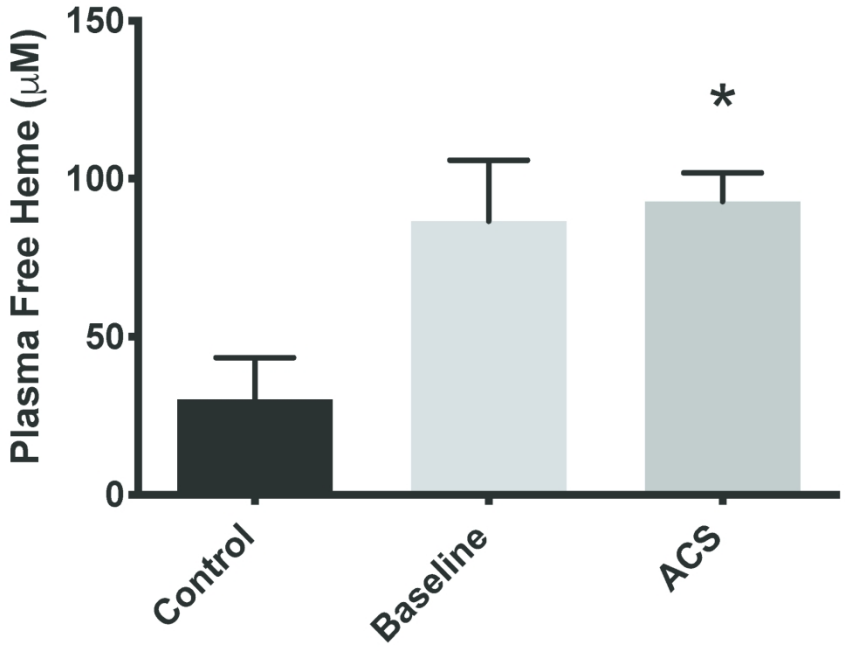
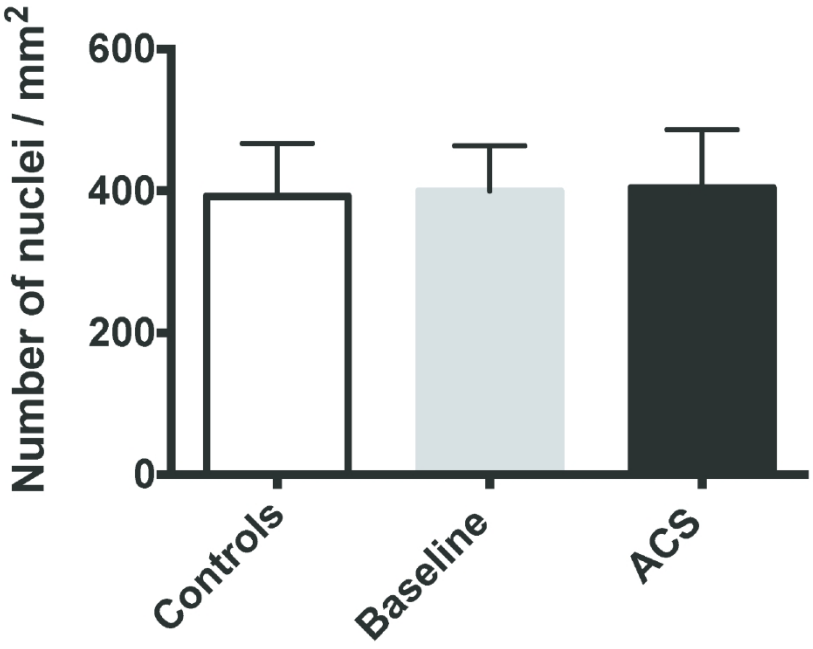


Supplemental Figure S1. Example illustrating method for quantifying monolayer disruption (extracellular space) using Image J software. Images show endothelial cells studied 48 hours after treatment with exosomes obtained from a subject with SCD during an episode of ACS. (a) Photomicrograph of endothelial monolayer with the overlay of immunoflourescent staining for VE-cadherin (green) and DAPI staining of the nuclei (blue). This image (and all images used for quantification) was obtained using the 10X objective to maximize the field size. (b) The micrograph was converted to a binary (black and white) image by applying the Image J automatic pixel intensity level threshold function. (c) The threshold was adjusted so that only the areas containing no cells are black. Thresholding is verified by side-by-side comparison of the binary and color images. A similar threshold was used for analysis of all images in an experiment (13 for the example shown here). The percentage of total image without any cells (black) was calculated using the Image J software using the command “analyze particles” in the Analyze menu. For the example shown, the area without cells was 9.3%. White arrows in (a) and (b) indicate spaces between the cells. Magnification bar represents 50 μm for all panels.



Supplemental Figure S2. Plasma free heme is increased in subjects with SCD compared to controls. Colorimetric analysis was performed to determine the amount of free heme in subjects' plasma. Subjects with SCD had increased plasma free heme, with no detectable difference between timpoints for subjects with SCD. (* = $p<0.05$ by unpaired t-test between control and ACS).



Supplemental Figure S3. EV treatment does not affect endothelial cell number. HMVEC-d cells were grown to confluence, treated with exosomes and stained with DAPI to visualize nuclei. We obtained multiple (n=6) low power (10X) micrographs from monolayers 48 hours following treatment with EVs from each subject/condition. DAPI-stained nuclei in each field were counted by an observer who was unaware of EV source and that was used to calculate number of nuclei (cells) per unit area. No significant differences were found.