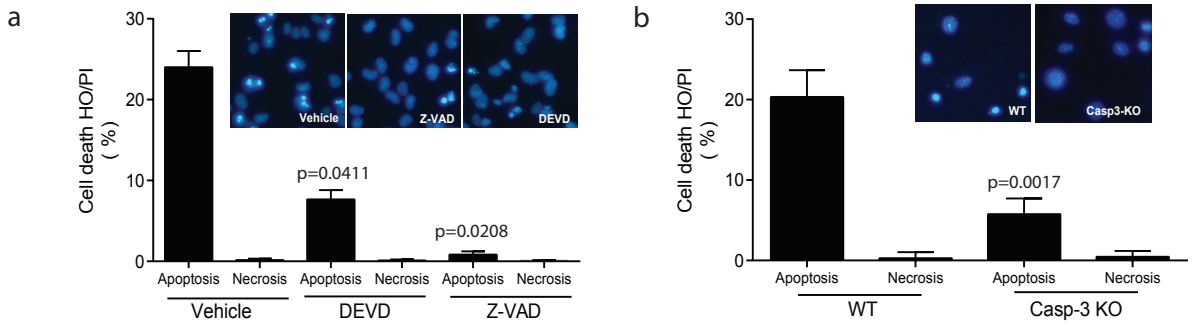
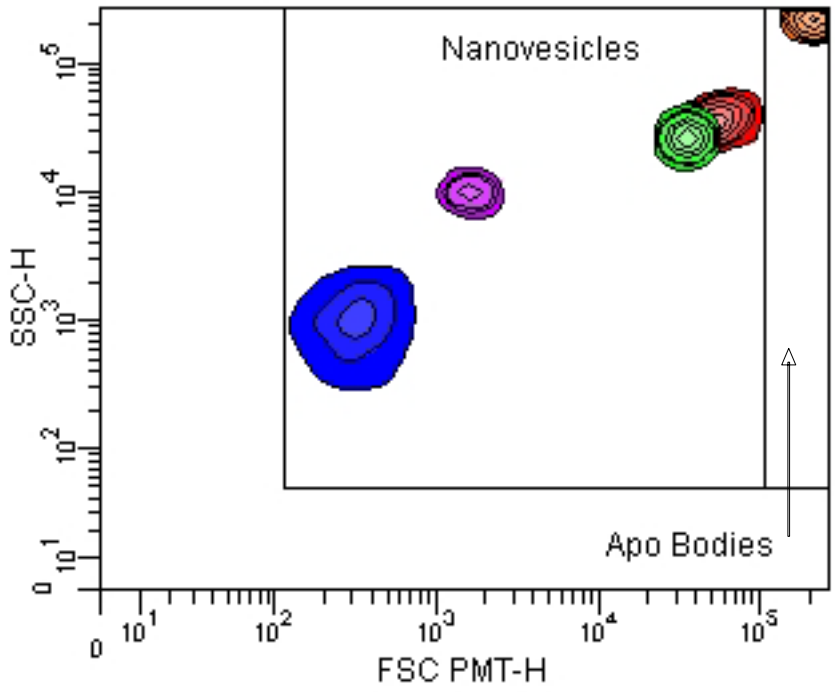
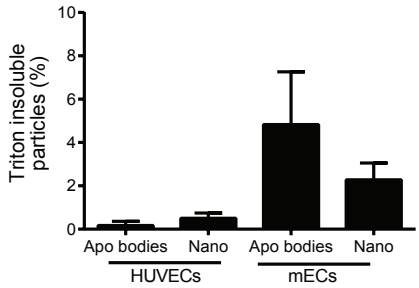


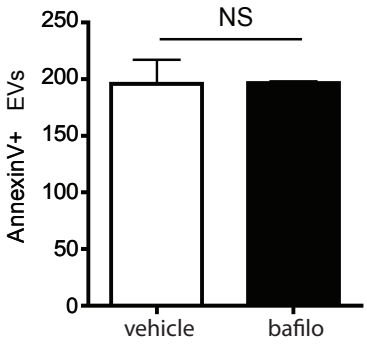
Supplementary figure 1. Serum deprivation significantly increased caspase-3 activation and the percentage of cells with chromatin condensation in the absence of cell membrane permeabilization a) Evaluation by Hoescht 33342 and Propidium iodide (HO/PI) staining of apoptotic or necrotic cells in serum-starved HUVECs exposed to vehicle, to the caspase-3 inhibitor Z-DEVD-fmk (DEVD) or to the pan-caspase inhibitor ZVAD-FMK (ZVAD). **b)** Evaluation by HO/PI staining of apoptotic or necrotic cells in serum-starved mEC from wild type mice (WT) or Caspase-3 knockout mice (Casp-3 KO). N=9, mean ± SEM, comparison with vehicle, t-test.



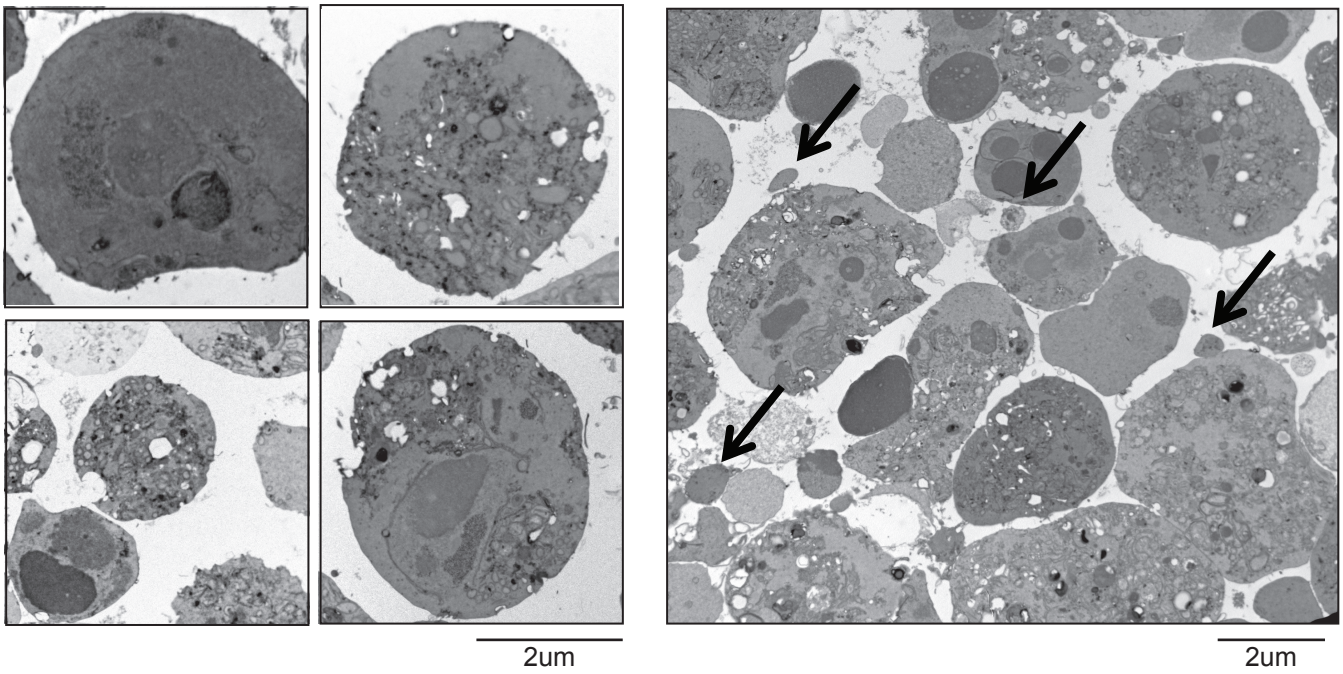
Supplementary figure 2. Small-particle flow cytometry size calibration. Acquisition of fluorescent Sky Blue microspheres of 40–90nm (mean 90 nm, in blue), 400-600nm (mean 450 nm, in pink), 700–900nm (mean 840 nm, in green), 1000nm (in red), 2500–4500nm (mean 3200 nm, in orange) diameter on a flow cytometer Canto II modified with a FSC-PMT small particles option. A exosome-like gate and an apoptotic body gate (Apo bodies) including respectively particles from 100 to 1000nm and larger than 1000nm in diameter based on the microsphere sizes (FSC-PMT-H) are presented and used to detect exosome-like vesicles and apoptotic bodies.



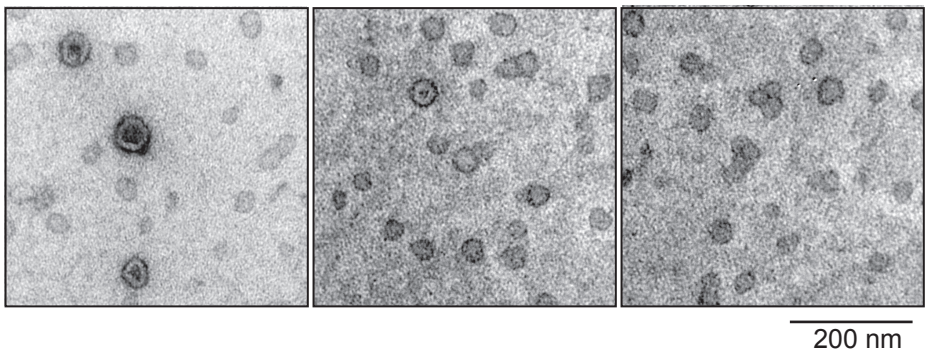
Supplementary figure 3. Characterization of small particles secreted by apoptotic ECs sensitive to detergent treatment
The vast majority of detected particles are sensitive to Triton-100 treatment. Percentages of Triton insoluble particles detected by high sensitivity flow cytometry after treatment of the supernatants with Triton (0.05%).



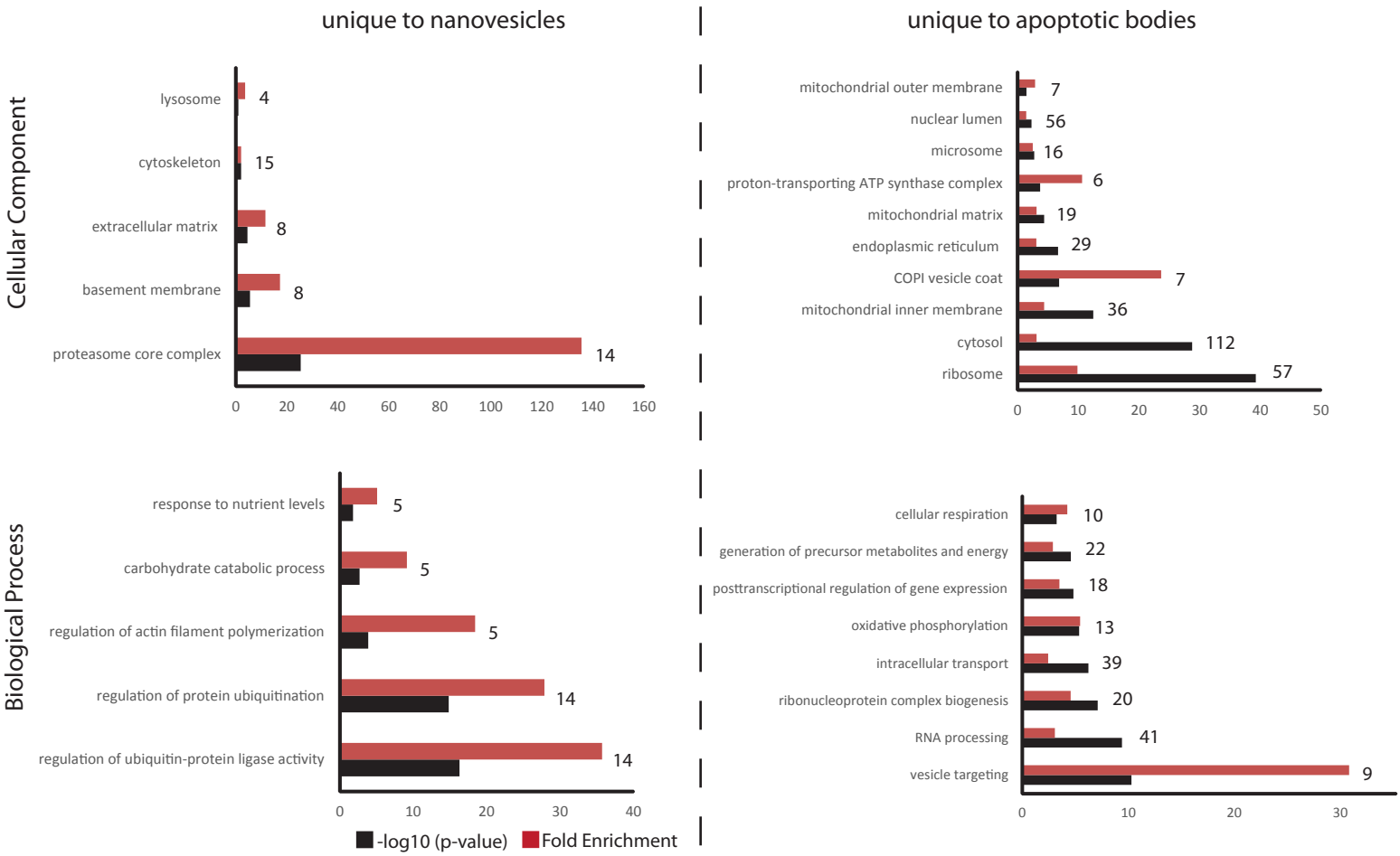
Supplementary figure 4. **Inhibition of autophagy with bafilomycin does not modulate exosome-like vesicle secretion by serum-starved ECs.** Flow cytometric quantifications of Annexin-V+ EVs secreted by HUVEC serum-starved for 4h and treated with the autophagy inhibitor bafilomycin A1 (5 nM) or vehicle.



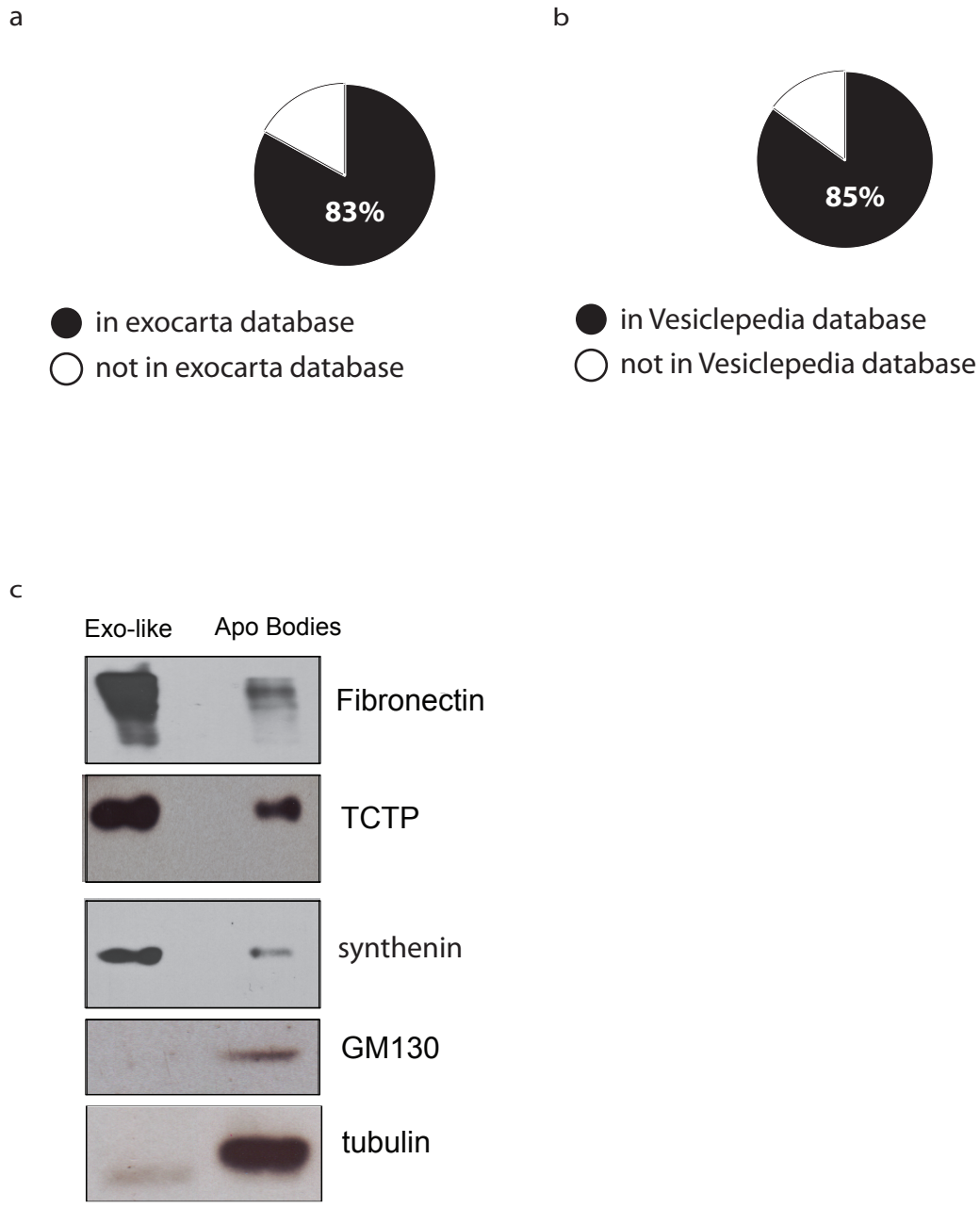
Supplementary figure 5. Electron micrographs of apoptotic bodies released by serum-starved HUVECs and isolated by sequential ultracentrifugation. The size range of the majority of vesicles is between 1 um and 5 um but smaller vesicles (arrow) between 0,25 um and 1 um, reminiscent of microparticles, are also present.



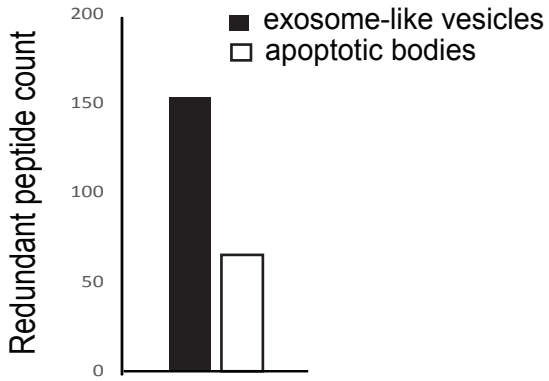
Supplementary figure 6. Electron micrographs of exosome-like vesicles released by serum-starved HUVECs and isolated by sequential ultracentrifugation.



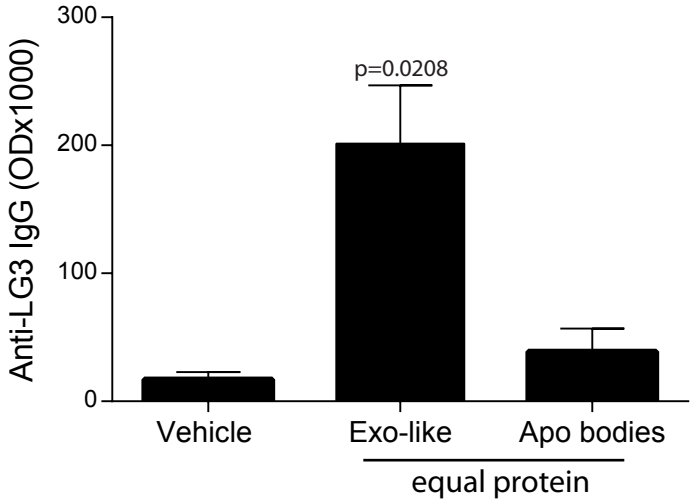
Supplementary figure 7. Gene ontology analysis of cellular components and biological processes for proteins unique to exosome-like vesicles and apoptotic bodies. In apoptotic bodies, terms related to mitochondria, ribosome or cellular respiration are enriched. In exosome-like vesicles, terms related to proteasome core complex, basement membrane or regulation of protein ubiquitination are enriched.



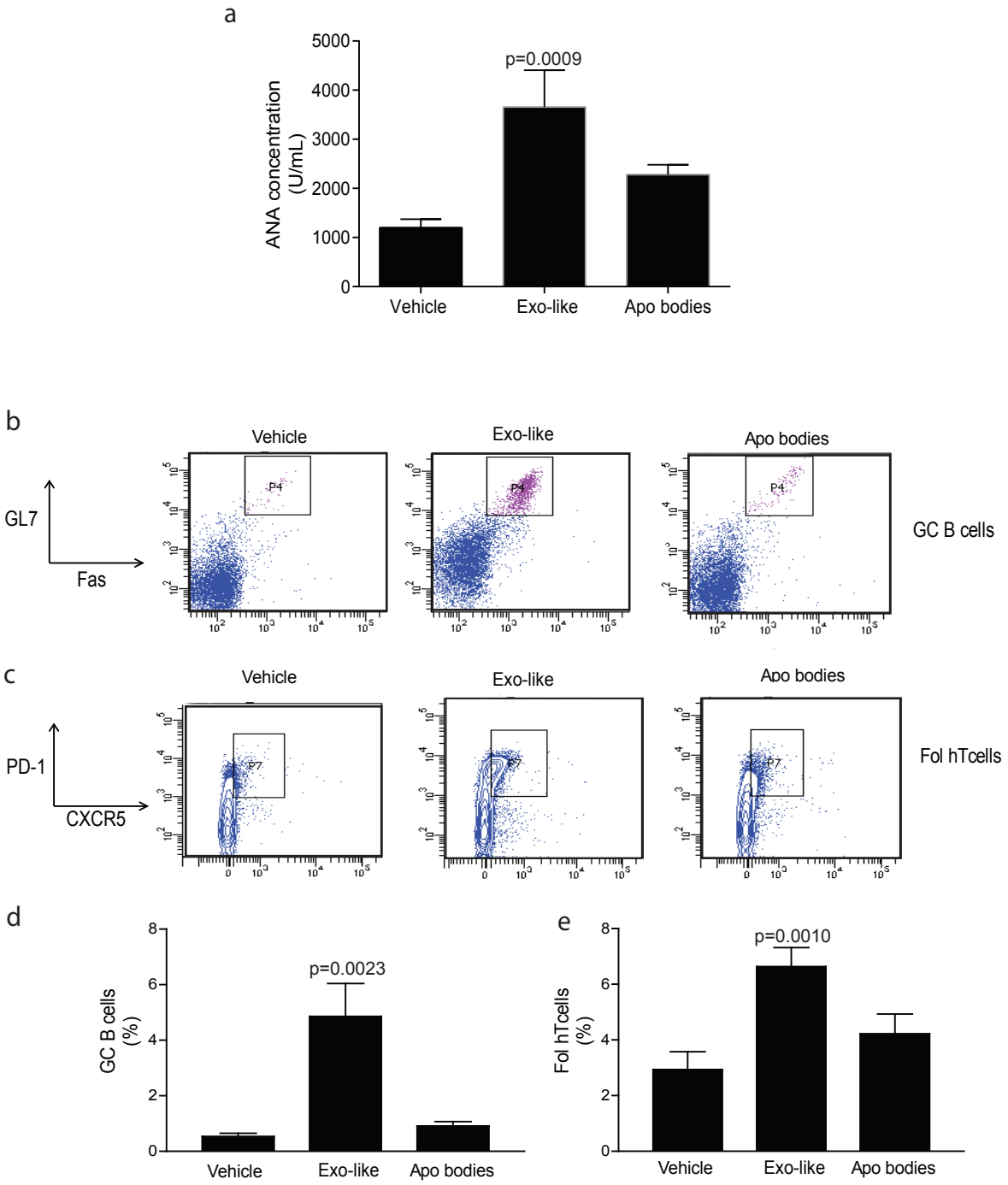
Supplementary figure 8. **Characterization of the presence of classical exosomal markers in the apoptotic exosome-like vesicles proteome a)** Analysis of the percentage of proteins identified in the proteome of apoptotic exosome-like vesicles and present in Exocarta database. **b)** Analysis of the percentage of proteins identified in the proteasome of apoptotic exosome-like vesicles and present in Vesiclepedia database **c)** Immunoblot analyses for different protein markers in apoptotic exosome-like vesicles and apoptotic bodies from medium conditioned by HUVECs serum starved for 4h.



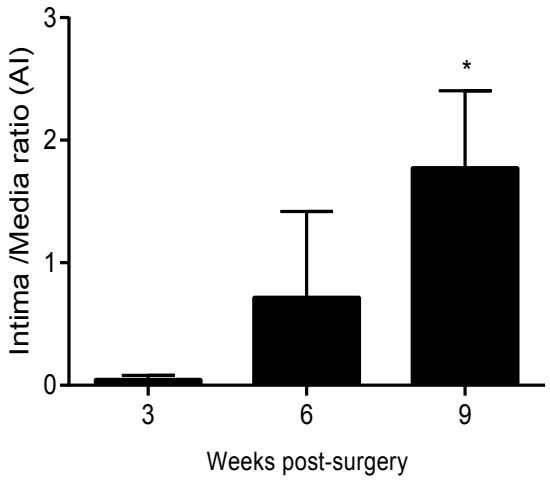
Supplementary figure 9. Redundant peptide count of perlecan in exosome-like vesicles and apoptotic bodies. Perlecan is enriched in exosome-like vesicles. Summary of peptides from the LG3 fragment detected in exosome-like vesicles and apoptotic bodies. Score = Mascot Score.



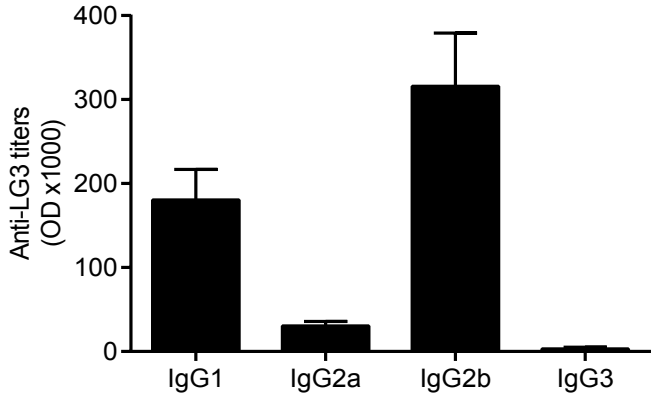
Supplementary figure 10. **Injecting equal amounts of proteins from preparations of apoptotic bodies and preparations of apoptotic exosome-like vesicles fail to unmask immunogenic activity in apoptotic bodies.** Anti-LG3 IgG titers in sera from WT mice after 3 weeks of i.v. injections with vehicle, or with equal protein amounts (0,6 ug / injection) of either exosome-like vesicles or apoptotic bodies from serum starved murine endothelial cells. n≥6 for each condition, mean ± SEM, t-test.



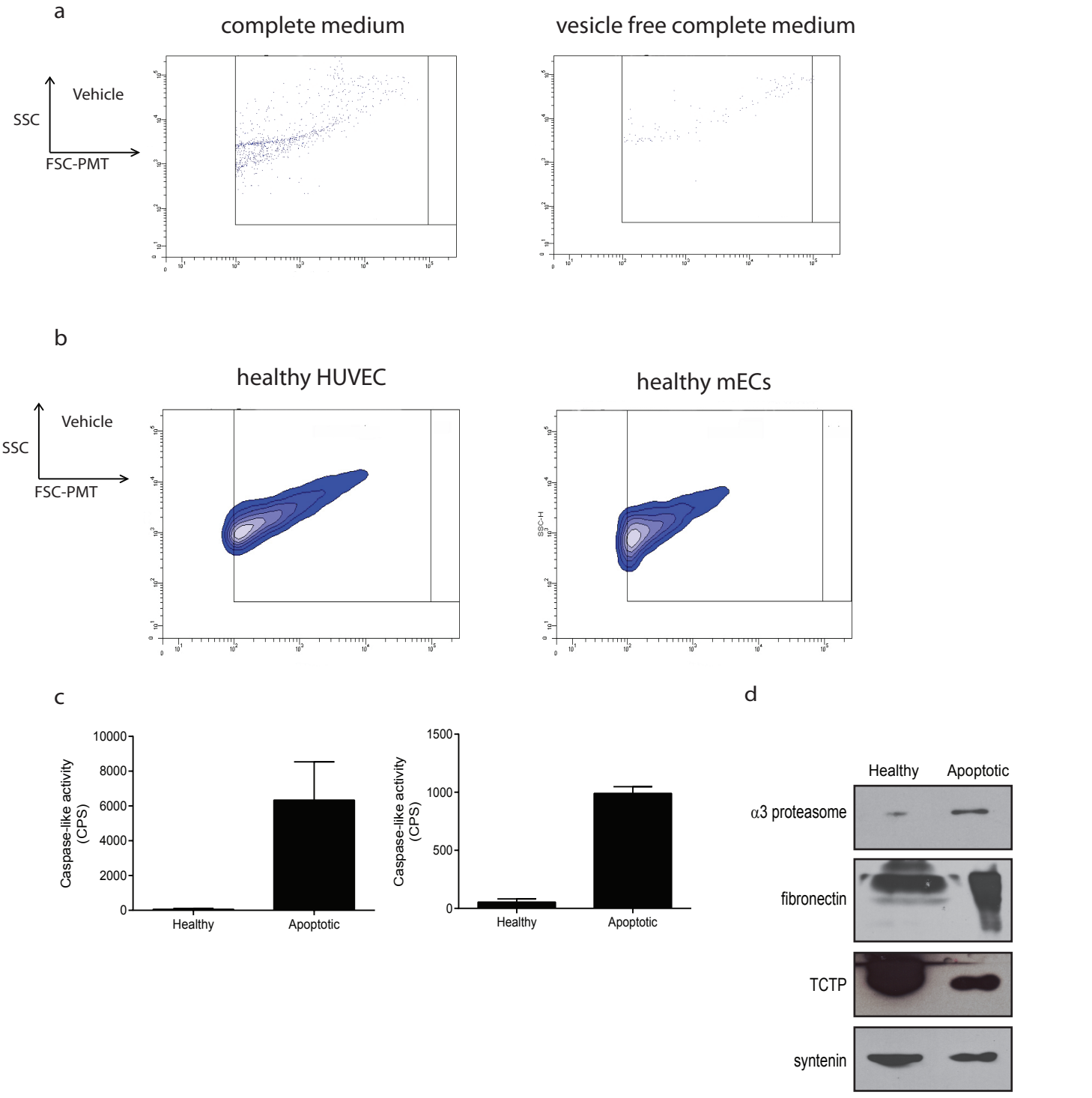
Supplementary figure 11. Apoptotic exosome-like vesicles, unlike apoptotic bodies, favor B cell responses and autoimmunity a) Concentration of anti-nuclear antibodies in sera from WT mice after 3 weeks of i.v. injections with vehicle, exosome-like vesicles or apoptotic bodies isolated from medium conditioned by serum starved murine endothelial cells. **b) and c)** . Flow cytometry analysis of germinal center (GC) B cell **b)** and follicular T helper cells **b)** B220+ B cells from spleen of mice after 3 weeks post-injection with vehicle, exosome-like vesicles or apoptotic bodies isolated from medium conditioned by serum starved murine endothelial cells were analyzed for GL7 and Fas expression. B220+GL7+Fas+ GC B cells among B220+ B cells are indicated by the gate **c)** PD-1+CXCR5+ Tfh cells among CD4+ cells was determined by flow cytometry and identified by the gate **d) and e)** Flow cytometry analysis of the percentages of splenic B220+GL7+Fas+ GC B cells **d)** or CD4+PD-1+CXCR5+ Tfh cells **e)** in mice after 3 weeks post-injection i.v. with vehicle, exosome-like vesicles or apoptotic bodies from serum starved murine endothelial cells. n≥6 for each condition. Data are pooled from 3 independent experiments. mean ± SEM, comparison with vehicle, t-test.



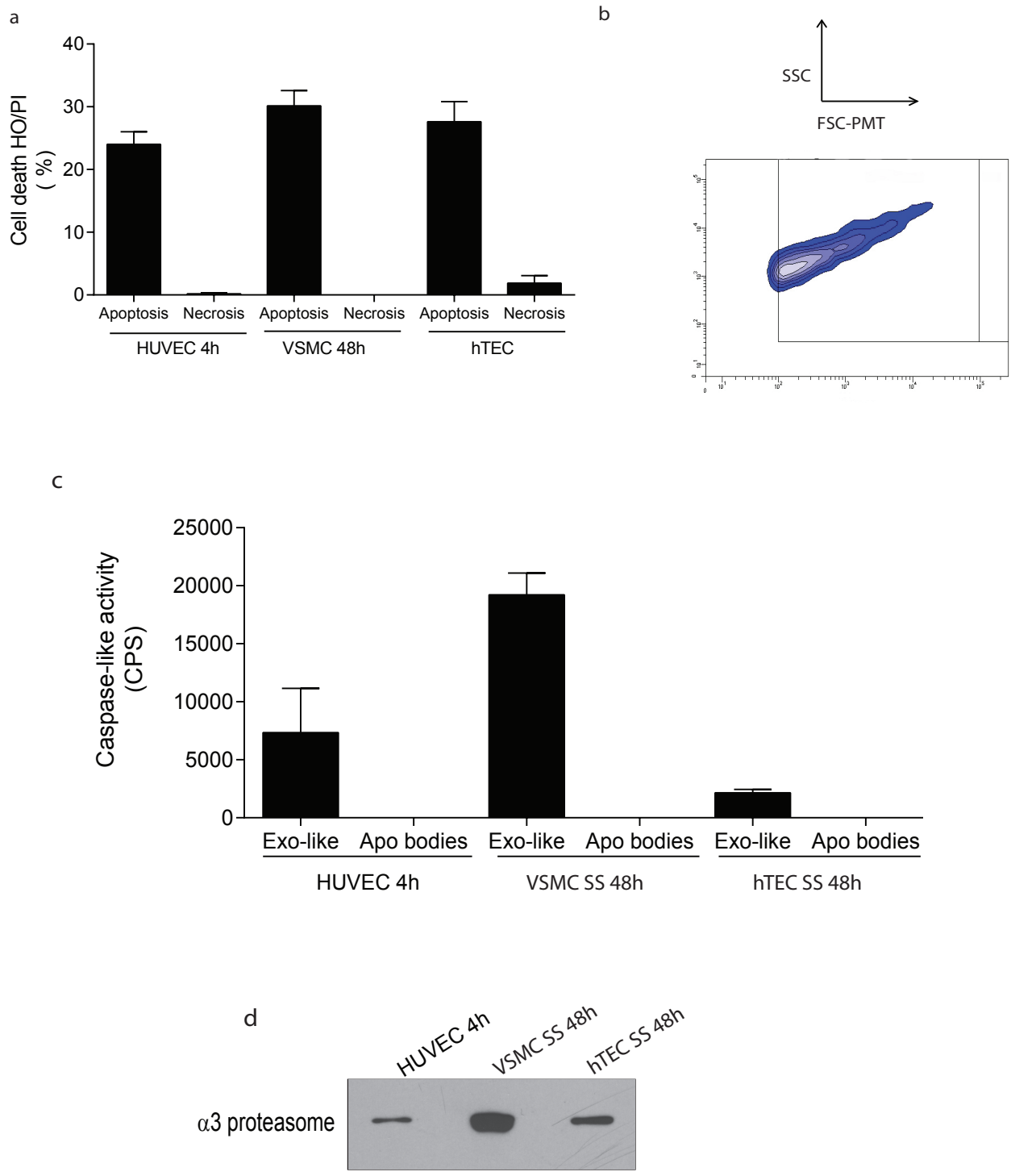
Supplementary figure 12. Intima/media ratio in murine allografts 3, 6, or 9 weeks after aortic transplantation. mean ± SEM, comparison with ungrafted, t-test.



Supplementary figure 13. Characterization of anti-LG3 IgG subclasses in sera harvested 3 weeks post-surgery from allografted mice injected with exosome-like vesicles for 3 weeks post-transplantation (n=10).

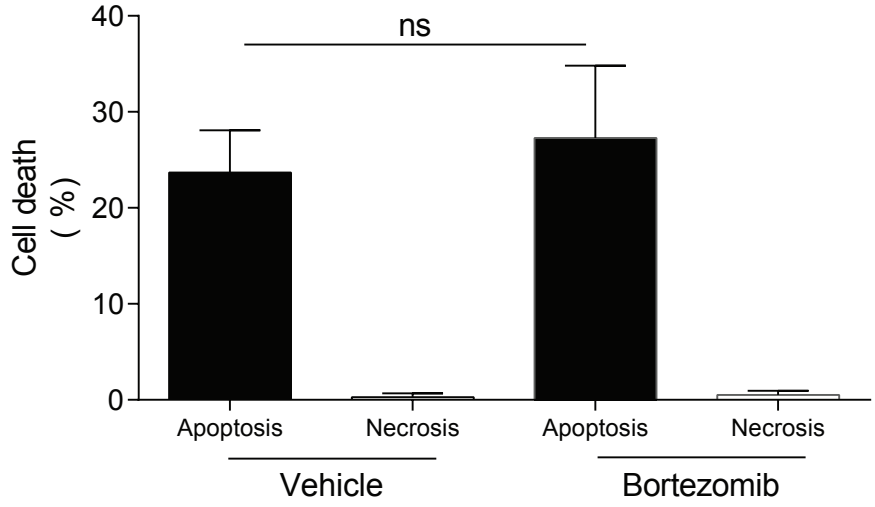


Supplementary figure 14. Healthy ECs secrete exosome-size vesicles lacking caspase-like proteasome activity. **a)**Representative FSC-PMT/SSC plot of the extracellular vesicles detected with fluorochrome-conjugated annexin-V in EBM-2MV complete medium **(left panel)** or vesicle free complete medium in which FBS was spun at 200 000xg to remove serum-derived vesicles **(right panel) b)** Representative FSC-PMT/SSC plot of the extracellular vesicles detected with fluorochrome-conjugated annexin-V in the supernatant of HUVECs incubated 4 h in normal medium (healthy HUVECs) **(left)** or mECs (healthy mECs) **(right) c)** Quantification of proteasome caspase-like proteolytic activity in extracellular vesicles isolated by ultracentrifugation at 200,000 xg from the supernatant of HUVECs **(left panel)** or mECs **(right panel)** serum starved for 4h (apoptotic) or incubated 4 h in normal medium (healthy). **d)** Immunoblot for different protein markers in extracellular vesicles isolated by ultracentrifugation at 200,000 xg from the supernatant of HUVECs serum starved for 4h (apoptotic) or incubated 4 h in normal medium (healthy).

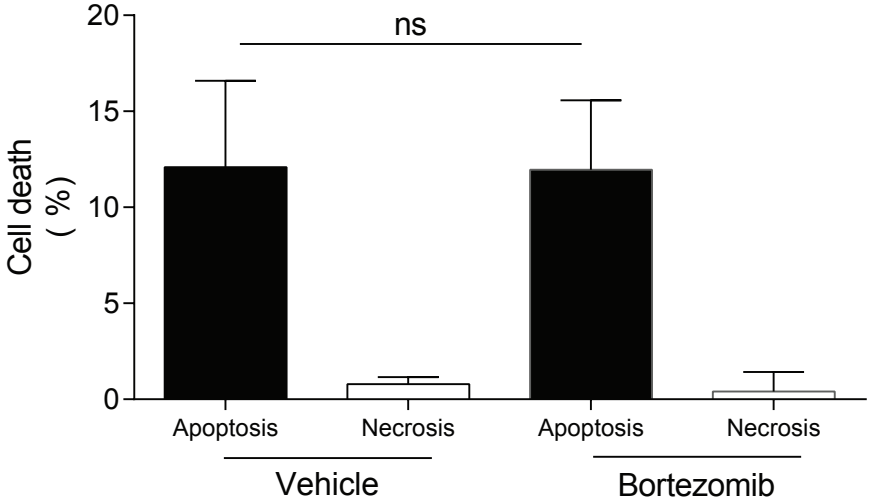


Supplementary figure 15. Apoptotic vascular smooth muscle cells and tubular epithelial cells also secrete active 20S proteasome in exosome-size EVs a) Evaluation by Hoescht and Propidium iodide (HO/PI) staining of apoptotic or necrotic cells in HUVECs, VSMCs, or hTECs serum starved for 4h, 48h and 24h respectively **b)** Representative FSC-PMT/SSC plot of the extracellular vesicles detected with fluorochrome-conjugated annexin-V in medium conditioned by VSMCs incubated 48 h in serum free medium **c)** Quantification of proteasome caspase-like proteolytic activity in extracellular vesicles isolated by ultracentrifugation at 50,000xg (apo bodies) or at 200,000 xg (exo-like) from medium conditioned by HUVECs, VSMCs or hTECs serum starved for 4h, 48h and 24h respectively. **d)** Immunoblot showing the detection of alpha 3 proteasome subunit in extracellular vesicles isolated by ultracentrifugation at 200,000 xg from medium conditioned by HUVECs, VSMCs, or hTECs serum starved for 4h, 48h and 24h respectively.

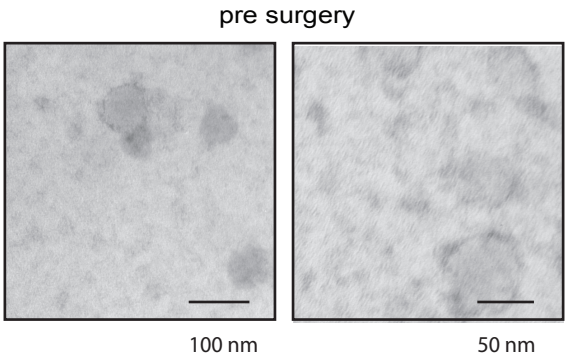
a



b



Supplementary figure 16. Bortezomib treatment does not affect cell death levels a) Percentages of HUVECs with increased chromatin condensation (apoptosis) and cell membrane permeabilization (necrosis), as evaluated by Hoescht and Propidium iodide staining, after exposure to serum free medium for 4h with vehicle DMSO or in presence of the proteasome inhibitor bortezomib **b)** Percentages of murine aortic endothelial cells with increased chromatin condensation (apoptosis) and cell membrane permeabilization (necrosis), as evaluated by Hoescht and Propidium iodide staining after exposure to serum free medium for 9h with vehicle DMSO or in presence of the proteasome inhibitor bortezomib. Data are pooled from 4 independent experiments. mean ± SEM, comparison with vehicle, ns= non-significant, t- test.



Supplementary Figure 17. **The proteasome is not detected in serum membrane vesicles isolated before surgery**. **Left panel:** Electron micrograph of serum membrane vesicles isolated pre surgery **Right panel:** Immunogold labeling for alpha 3 proteasome subunit in serum membrane vesicles isolated pre surgery. No labeling was present.