Figure S1. Characterization of mEVs using flow cytometry. A whole blood sample obtained from a splenectomised β-thalassaemia/HbE patient was analysed for mEVs using flow cytometry. (A) The sample was mixed with nano polystyrene beads (0.79 μm beads in R1 region and 1.32 μm beads in R2 region) to identify the mEV population in the R3 region, the platelet population in the R4 region, and the RBC population in the R5 region. (B) The sample was mixed with TruCount beads (R6 region) to calculate the absolute number of mEVs. (C) Annexin V positive mEVs were identified in the R7 region. MEVs, medium extracellular vesicles.

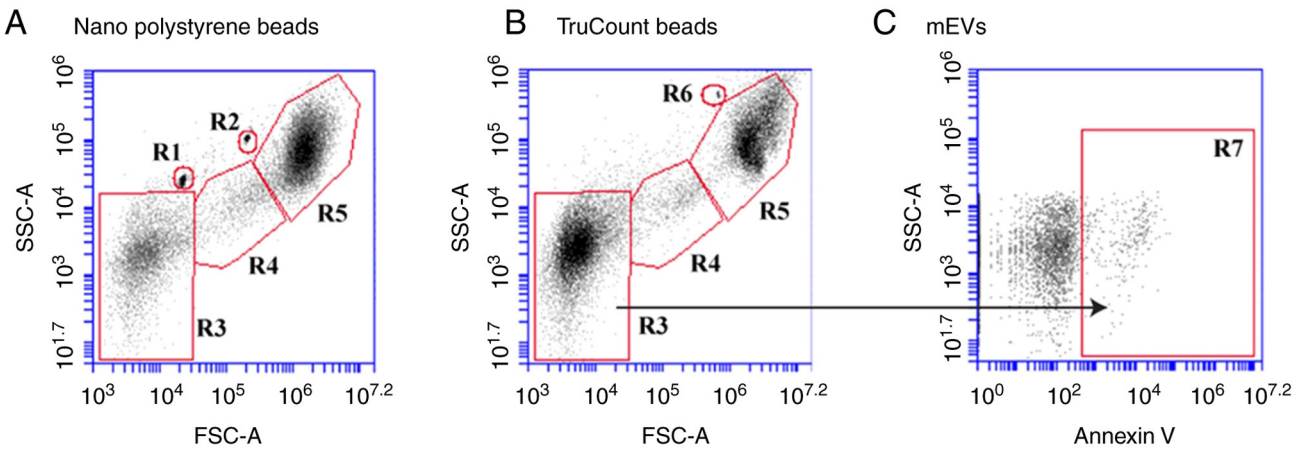

Figure S2. Representative X-ray film of western blot analysis. (A) Phosphorylated eNOS at Ser1177 (p-Ser1177), phosphorylated eNOS at Thr495 (p-Thr-495), total eNOS and b-actin in untreated HPAECs and HPAECs treated with either VEGF, mEVs from healthy subject (N), or from β-thalassaemia/HbE patient (BE), respectively. A single membrane was used to probe for eNOS, phosphorylated eNOS (at p-Ser1177 and p-Thr-495), and b-actin. The membrane was cut to separately analyse eNOS (140 kD) and β-actin (45 kD). After probing for total eNOS, the eNOS section of the membrane was stripped and sequentially re-probed for p-Ser1177, followed by stripping and re-probing for p-Thr-495. The β-actin section of the membrane was similarly stripped and re-probed each time after probing for total eNOS and each phosphorylated eNOS form. Both sections were then detected together in the final analysis. (B) α-globin in mEVs from healthy subjects (n=3) and from β-thalassaemia/HbE patients (n=3). ENOS, endothelial nitric oxide synthase; HPAECs, human pulmonary artery ECs; mEVs, medium extracellular vesicles; N, healthy subjects; BE, β-thalassaemia/HbE patient.