SUPPLEMENTARY INFORMATION

Real-time imaging of VCAM-1 mRNA in TNF-α activated retinal microvascular endothelial cells using antisense hairpin-DNA functionalized gold nanoparticles

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Running Title: Real-time Imaging of mRNA expression levels in retinal endothelial cells

Key Words: VCAM-1, mRNA, microvascular endothelial cells, imaging, gold nanoparticle.

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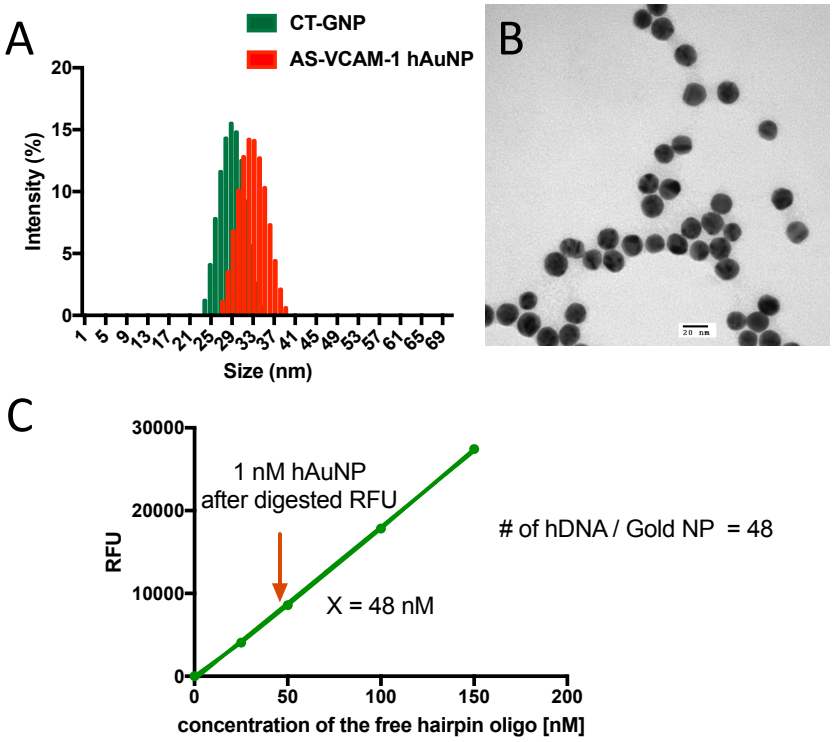

Figure S1: Characterization of hAuNP after their synthesis. (A) Increase in hydrodynamic diameter of the citrate caped gold nanoparticle (green) after addition of the hairpin DNA oligonucleotide (red). (B) Transmission electron microscopy images of the AS-VCAM-1 hAuNP showing the mono-dispersity of the probe. (C) Calculation of number of hairpin DNA oligonucleotides on the surface of each gold nanoparticle. Due to the quenching properties of gold, hAuNP are non-fluorescent. hAuNP were digested with DTT releasing the hairpin DNA oligonucleotide which is no longer quenched. The concentrations of hairpin DNA oligonucleotide samples were determined using standard fluorescence measurements and linear regression analysis.

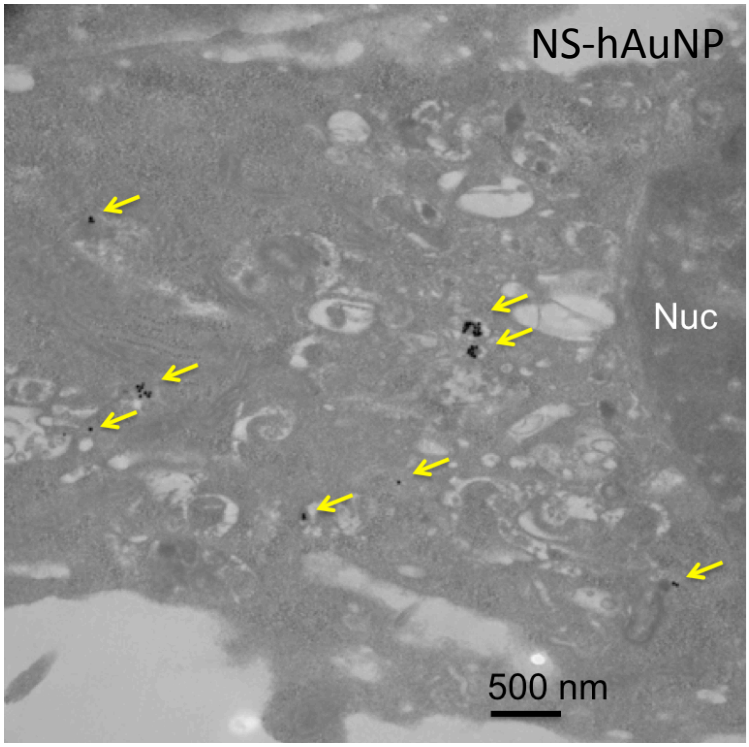

Figure S2: Detection nonsense hAuNP (NS-hAuNP) localized in MRMECs using transmission electron microscopy imaging (TEM). TEM micrograph shows clusters of hAuNP localized in either endosomes or lysosomes throughout the cytoplasm in TNFα treated MRMECs.

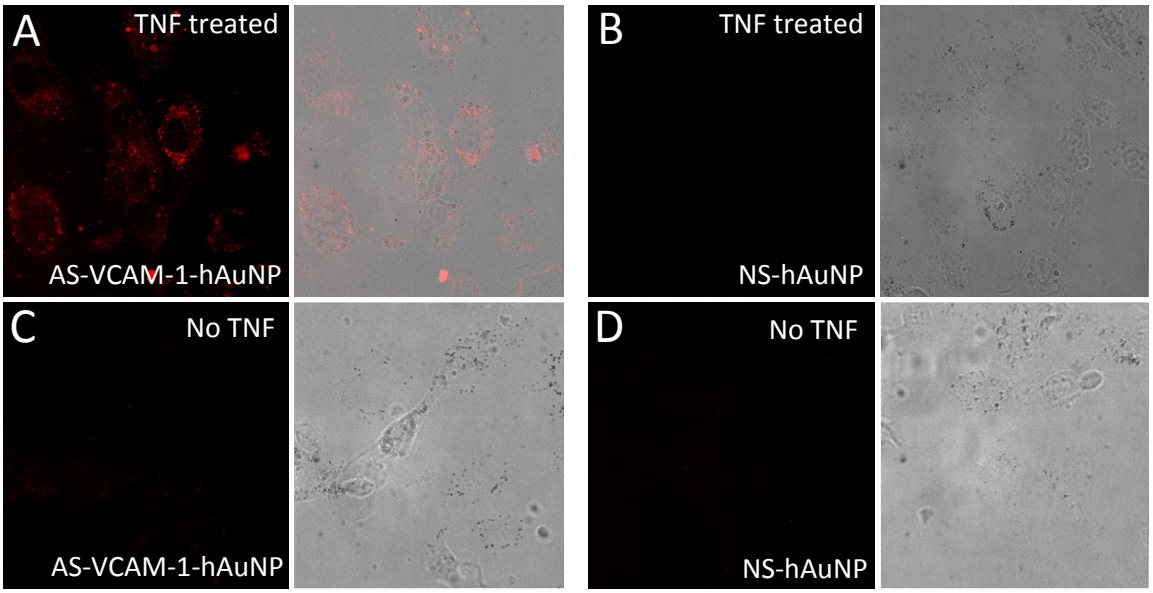

Figure S3: Confocal imaging of living MRMECs using hAuNP. Cells were cultured on chambered microscope slides and treated with TNF-α or vehicle plus AS-VCAM-1 hAuNP or NS-hAuNP in complete growth medium. They were incubated for 3 days, these media were removed and fresh medium (without phenol red) was added to each culture for confocal imaging. AS-VCAM-1 and NS-hAuNP were taken up by the cells and retained over the time course of the experiment (A-D). (A) Strong fluorescence was detected in AS-VCAM-1 hAuNP treated MRMECs activated with TNF-α. (B, C and D) Only minimal fluorescence was detected in the other cultures.