Supporting information for

High Density Lipoprotein Nanoparticles Deliver RNAi to Endothelial Cells to Inhibit Angiogenesis

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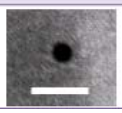
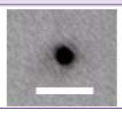
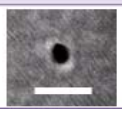
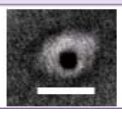
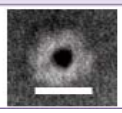
Table S1. Characterization data for nano-constructs at step end-points outlined in the synthetic scheme for chol-ON-HDL NP conjugates.

	AuNP	ApoA1+AuNP	HDL NP	HDL NP + NS	HDL NP + AS
Size (nm)	6.15±1.34	9.17±2.55	15.09±3.64	19.92±4.97	17.24±4.84
UV-Vis λmax (nm)	519	523	526	527	527
ζ-potential (mV)	-14.3±4.5	-34.5±5.67	-37.0±0.93	-39.7±8.16	-39.5±4.44
Mean ApoA1:AuNP molar ratio	-	2.87±0.21	2.74±0.17	2.22±0.61	2.14±0.73
Mean RNA:AuNP molar ratio	-	-	-	17.01±3.19	15.52±2.91
TEM (20 nm scale-bar)					

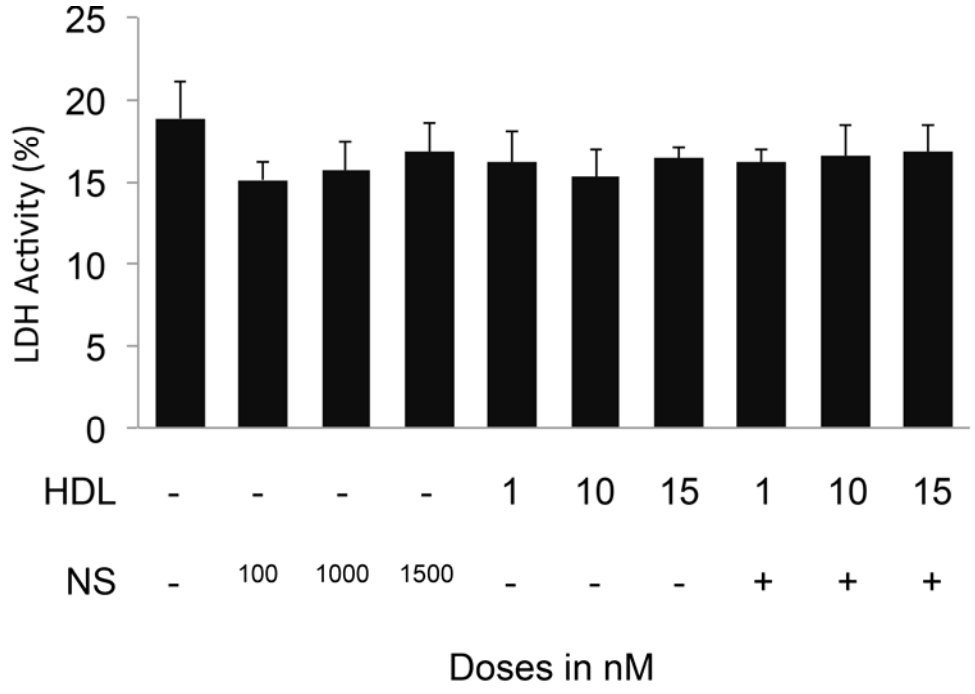


Figure S1. LDH assay for cytotoxicity. Cytotoxicity in HUVECs is measured as amount of released lactate dehydrogenase (LDH) activity upon treatment with HDL NPs (HDL), free NS ONs (NS) and their conjugates (HDL x nM/ NS+). HDL NPs and NS-HDL NPs (HDL x nM/ NS+) treatments are 1, 10, 15 nM (final). Free NS ONs are 100, 1000, 1500 nM (final). No significant change in cytotoxicity was measured after the various treatments as compared to untreated control (far left).

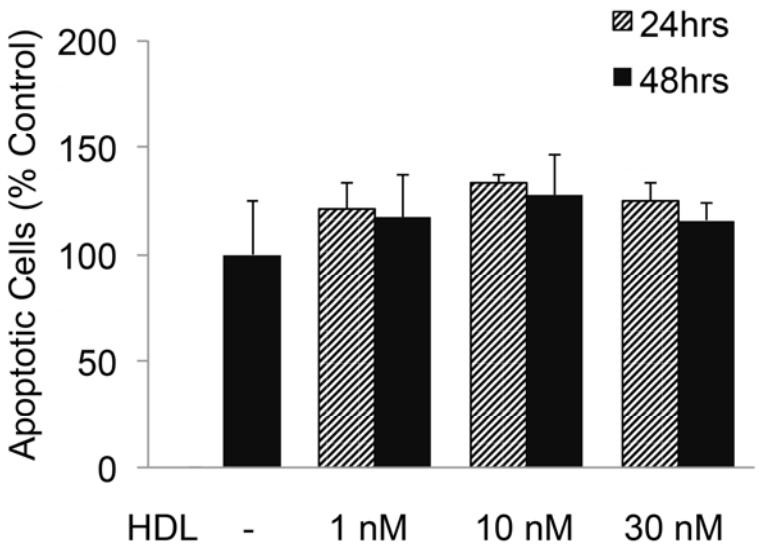


Figure S2. Apoptosis in cultured endothelial cells after HDL NP exposure. Apoptotic response in HUVECs mediated by varying doses of HDL NPs was measured as Annexin V positive fraction of cell population identified via flow cytometry. No significant difference was measured between the untreated (far left) and treated samples at 24 or 48 hrs.

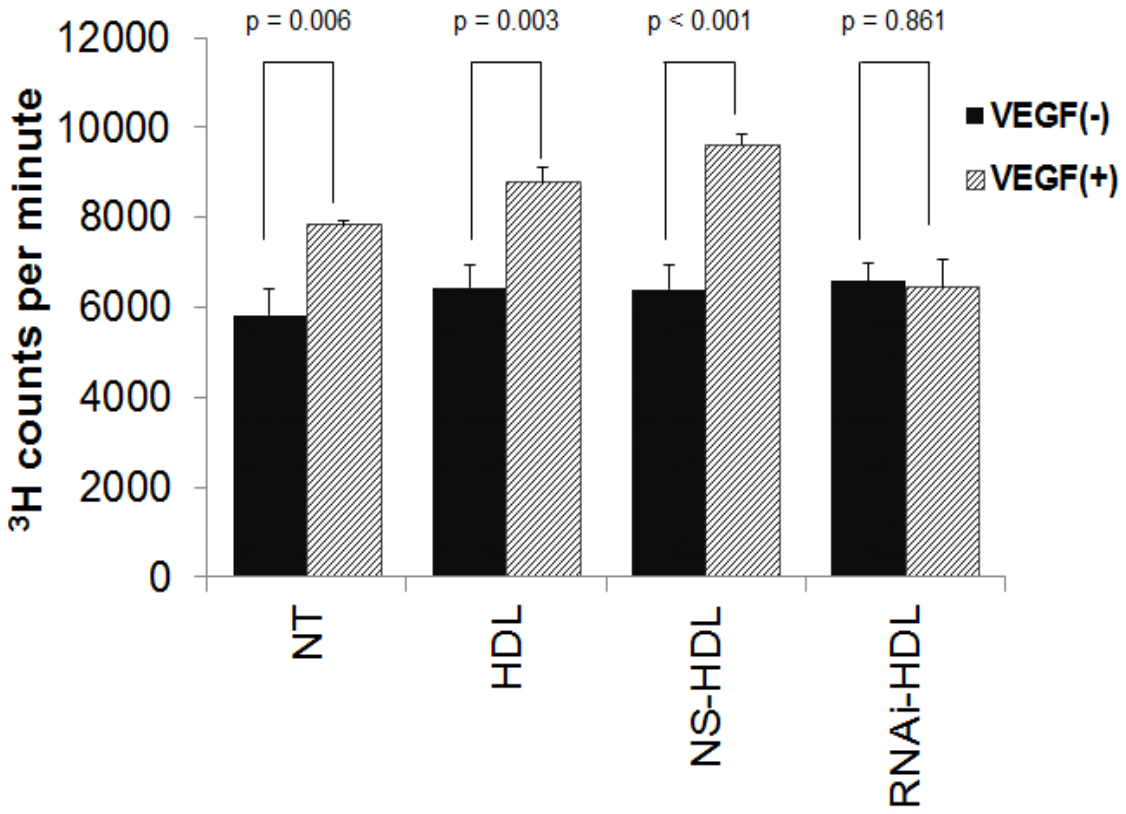


Figure S3. RNAi-HDL NPs reduce VEGF dependent proliferation in cultured HUVECs. Proliferation measured as ^{3}H thymidine incorporation is not significantly different in presence or absence of VEGF-A in the RNAi-HDL NPs (RNAi-HDL) treatment.

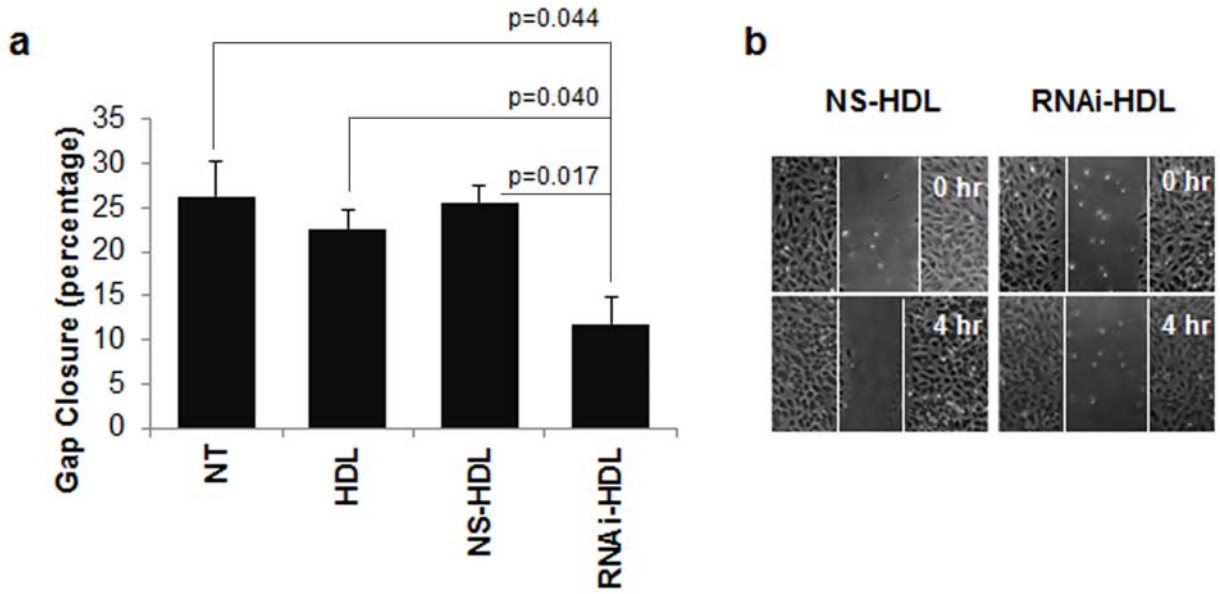


Figure S4. RNAi-HDL NPs inhibit VEGF induced chemotaxis in HUVECs. Chemotaxis, assayed as the percent gap closure is significantly reduced upon RNAi-HDL NPs treatment. Plot of averaged gap closure percentage (a) and representative images (b) are shown.

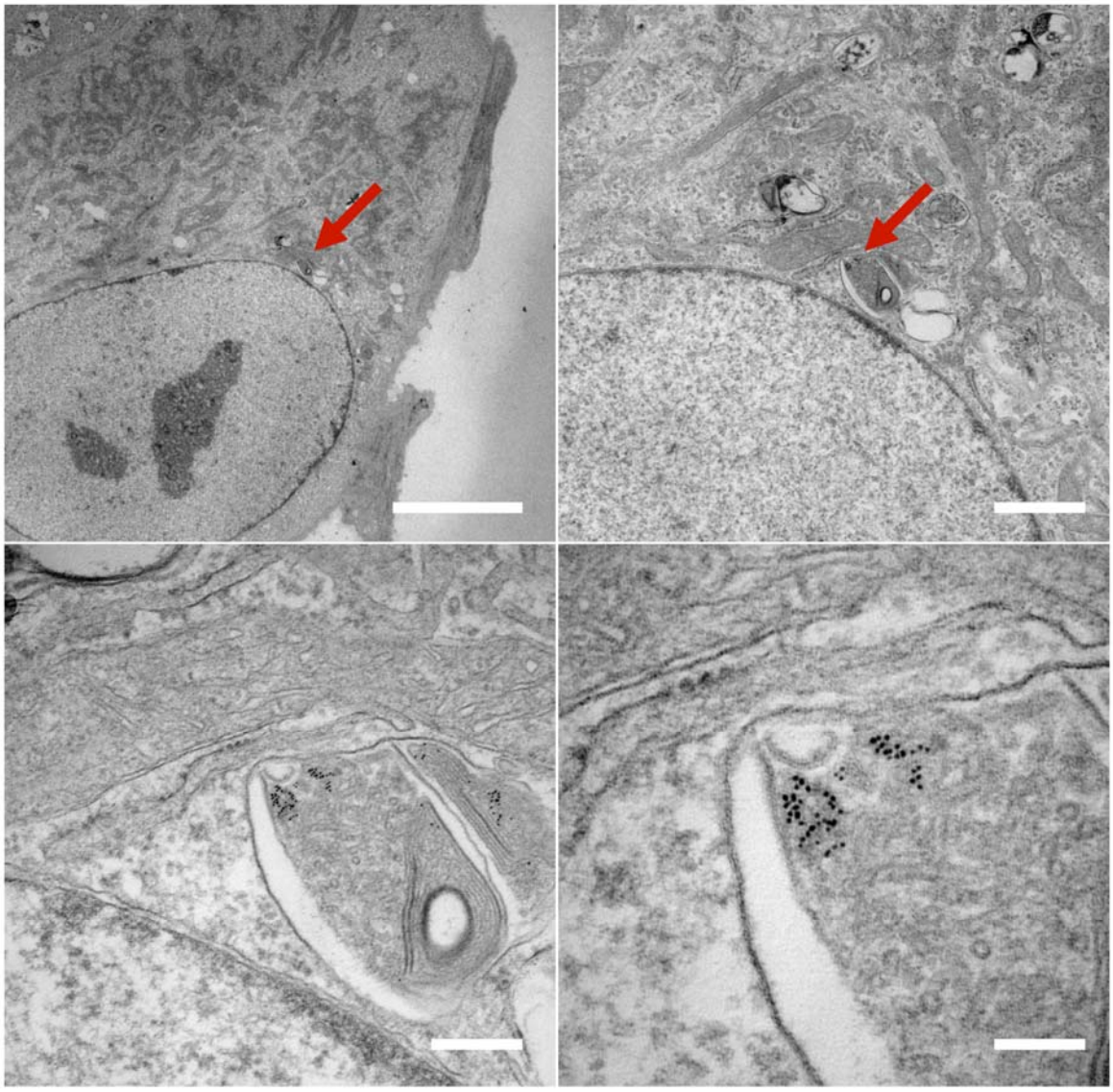


Figure S5. NS-HDL NP uptake by HUVECs: localization to the vesicular compartments. Transmission electron micrographs of NS-HDL NPs treated HUVECs are shown. Gold NPs forming the core of the NS-HDL NPs are electron dense and appear as black spheres inside vesicular compartments of the treated HUVECs (scale bars clockwise from top-left, 5 μm, 1 μm, 100 nm, and 200 nm).

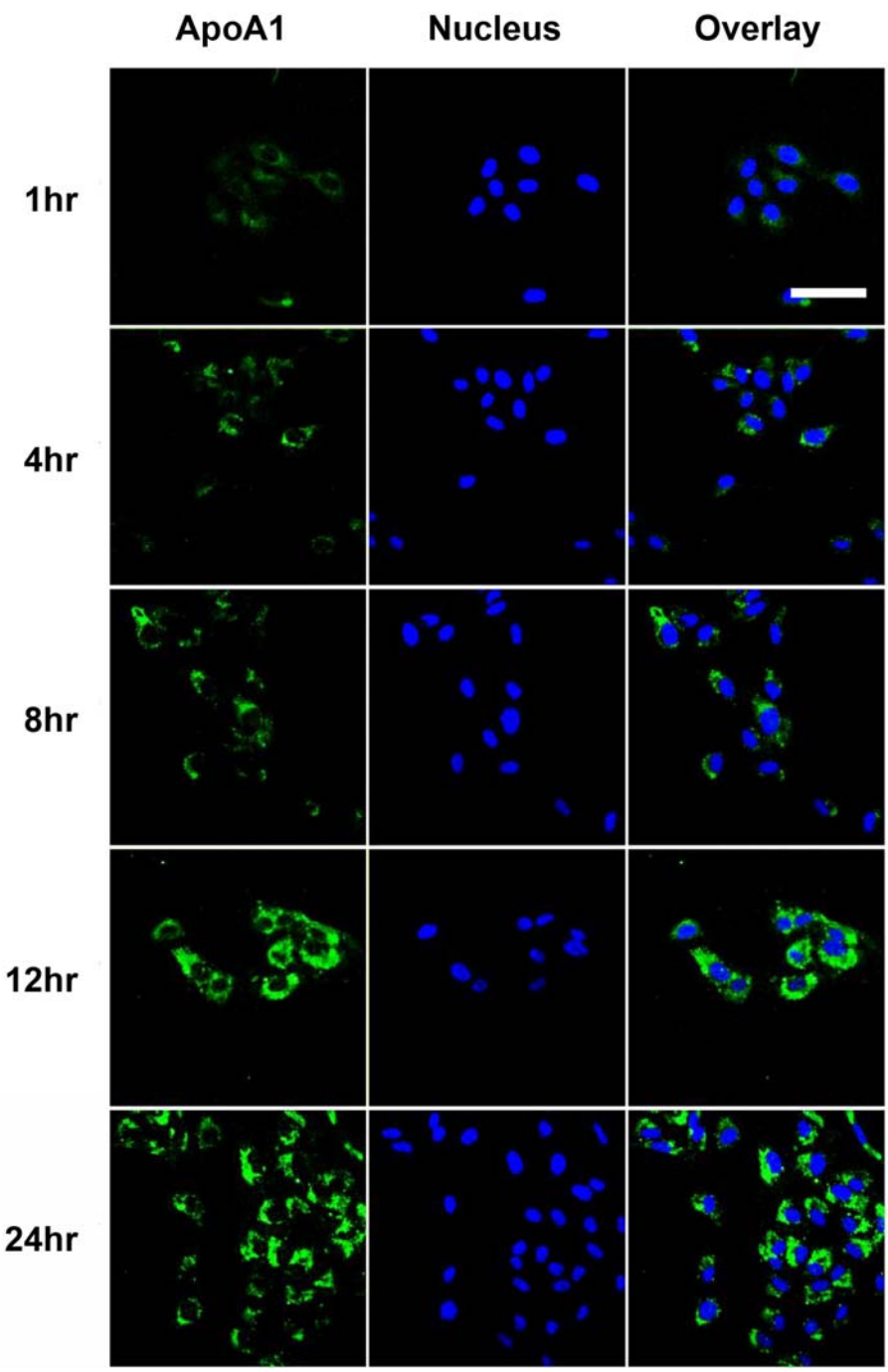


Figure S6. The protein component of NS-HDL NPs (apolipoprotein A1) localizes to the cytosol by 4 hrs and can be detected as late as 24 hrs post treatment. HUVECs treated with Alexa Fluor 488 tagged ApoA1 containing NS-HDL NPs, fixed at indicated time points are imaged via confocal microscopy (scale bar, 30 μm).

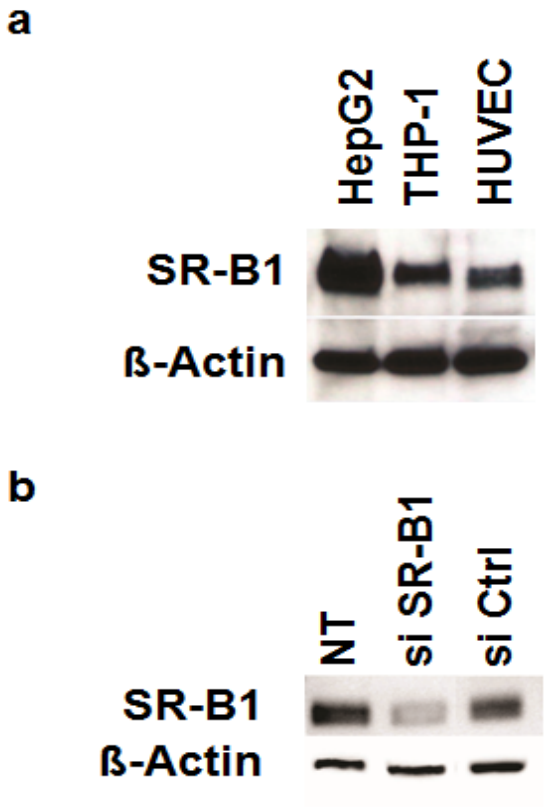


Figure S7. HUVECs express SRB1. (a) Western blots for SRB1 with ß-actin as loading control. HUVECs express SRB1 at levels comparable to that of HepG2 and THP-1 cell lines. (b) Transient SRB1 knockdown with validated siRNA (using Lipofectamine RNAiMax reagent) 48 hours post treatment.

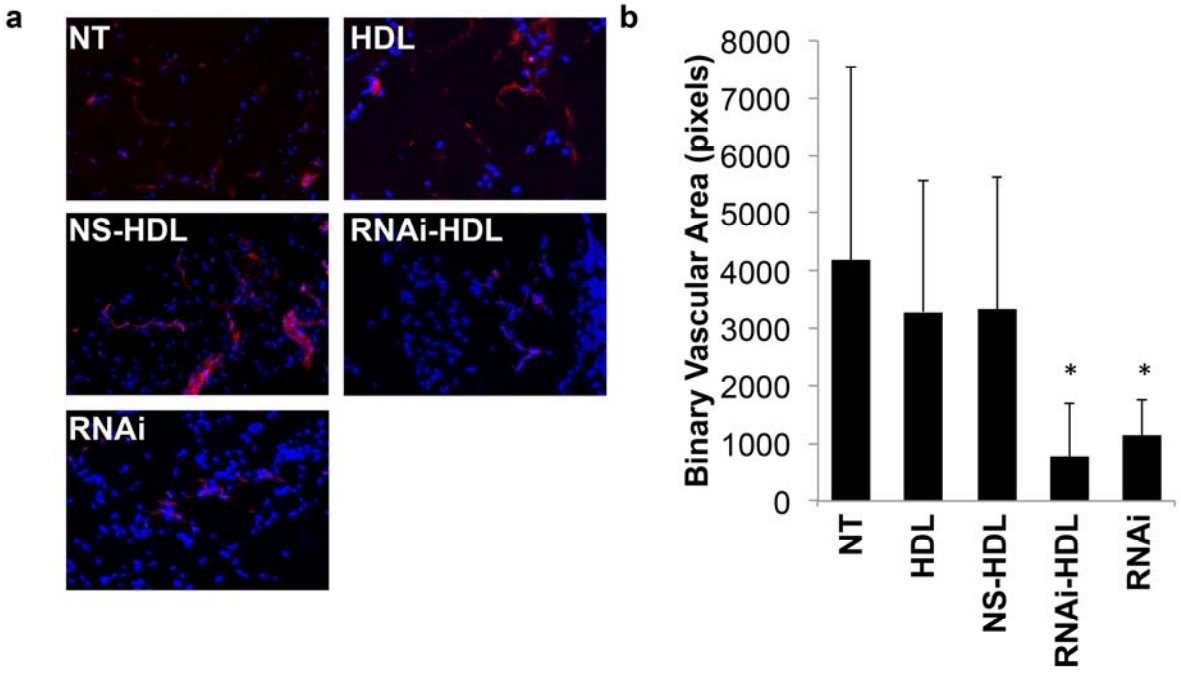


Figure S8. HUVECs treated with RNAi-HDL NPs prior to Matrigel plug implantation. HUVECs pre-treated in culture with PBS (NT), HDL NPs (HDL), NS-HDL NPs (NS-HDL), RNAi-HDL NPs (RNAi-HDL) and free RNAi (RNAi) are incorporated into Matrigel plugs, which are implanted into median abdominal area of mice to assess angiogenesis. The vasculature is visualized by CD31 staining (red) and cell nuclei are stained with DAPI (blue). Representative fluorescent images (a) and quantification plot (b) (average binary vascular area) are shown. Significant differences in average binary vascular area are observed in RNAi-HDL NPs treatment and free RNAi treatment. Error bars (standard deviation) and statistically significant differences (Student's t-Test, with $p < 0.05$) are marked with asterisks.

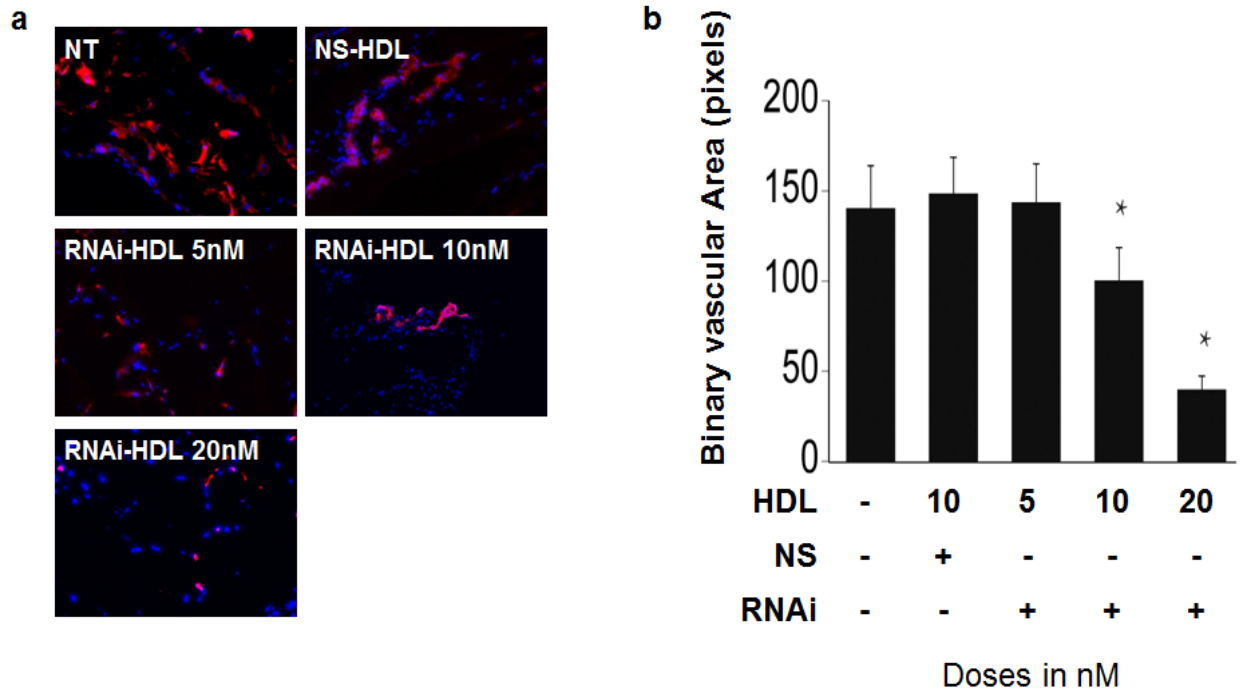


Figure S9. Local delivery of RNAi-HDL NPs to Matrigel. PBS (NT), NS-HDL NPs (NS-HDL, 10 nM final concentration), RNAi-HDL NPs (RNAi-HDL, 5, 10, 20 nM final concentrations) are incorporated into Matrigel plugs, which are implanted into median abdominal area of mice to assess angiogenesis**.** The vasculature is visualized by CD31 staining (red) and cell nuclei are stained with DAPI (blue). Representative fluorescent images (a) and corresponding calculated average binary vascular area plot (b) are shown. Significant differences in average binary vascular area are observed in RNAi-HDL NPs 10, 20 nM treatments. Error bars denote standard deviation and statistically significant differences (Student's t-Test, $p < 0.05$) are marked with asterisks.

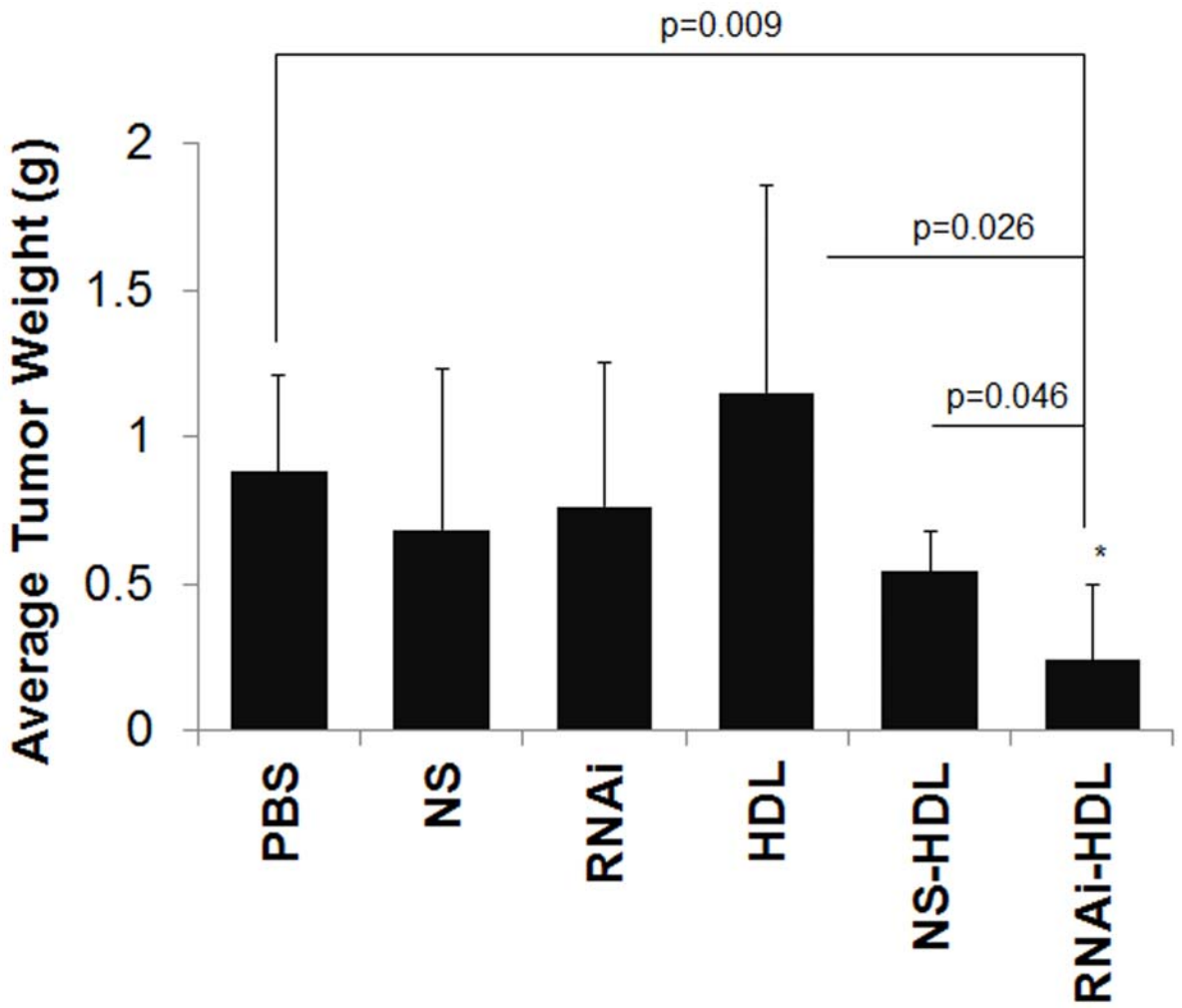


Figure S10. Systemic administration of RNAi-HDL NPs inhibited tumor growth in subcutaneous LLC-1 allograft tumors resulting in lower final tumor weights. Average murine LLC-1 tumor weights upon conclusion of study, for mice treated with PBS, NS ONs (NS), RNAi, HDL NPs (HDL), NS-HDL NPs (NS-HDL) and RNAi-HDL NPs (RNAi-HDL) are shown. RNAi-HDL NPs treated tumors show a significant reduction in average tumor weight.

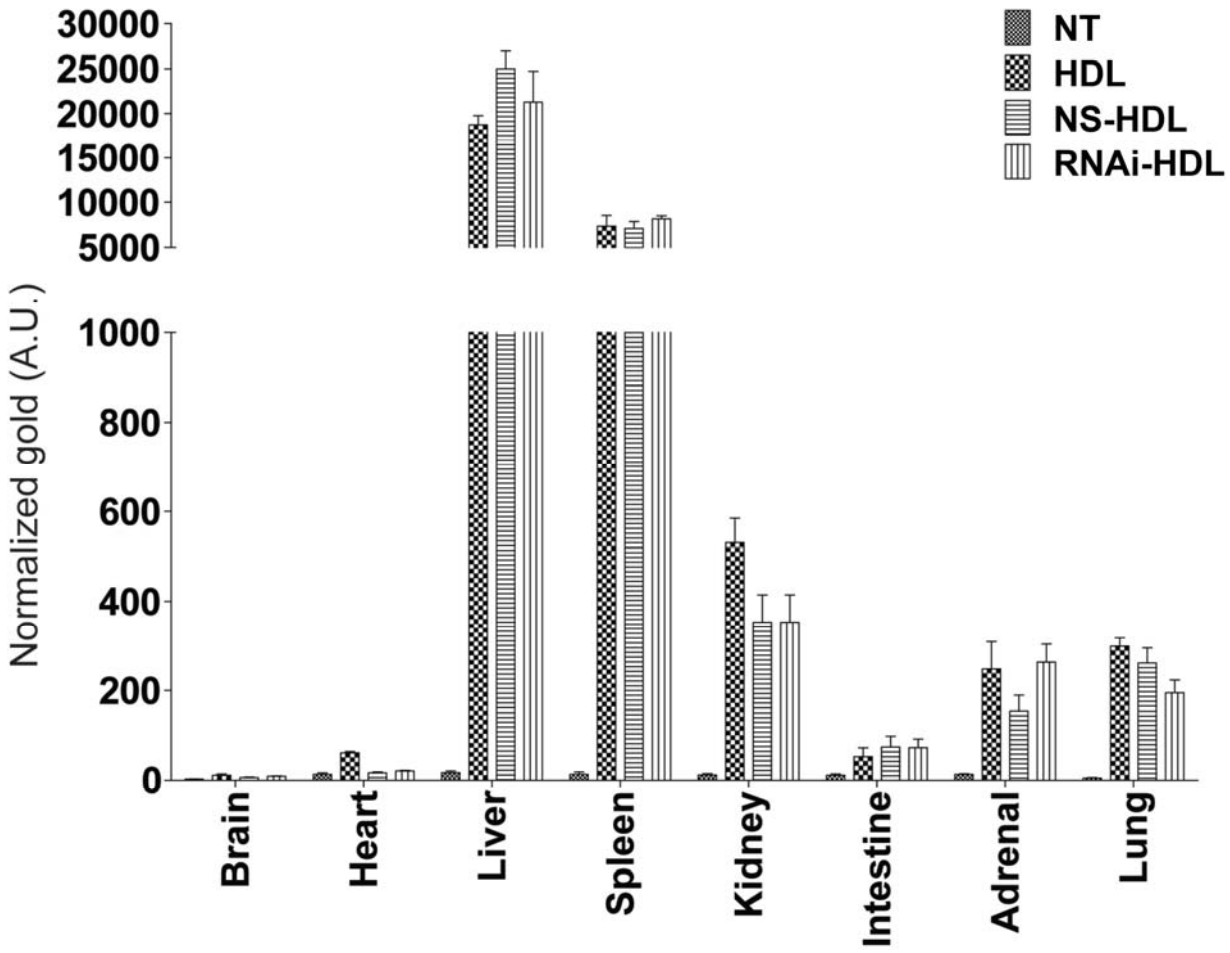


Figure S11. Biodistribution of HDL NPs and their conjugates with NS ONs and RNAi upon systemic administration (via tail vein injection). ICP-MS plots for gold content normalized per mg of dry tissue weight for various organs from mice that were systemically administered HDL NPs (HDL) and conjugates with RNAi (RNAi-HDL) and NS ONs (NS-HDL) are shown.

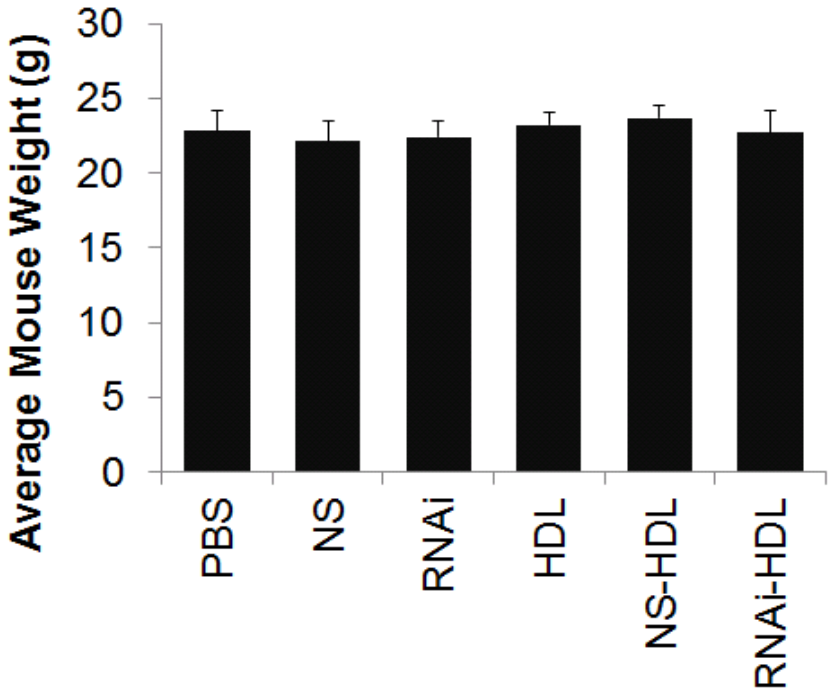


Figure S12. Mice treated with RNAi-HDL NPs display no significant body weight differences as compared to controls after a 9-day treatment period.