Targeting Extracellular DNA to Deliver IGF-1 to the Injured Heart

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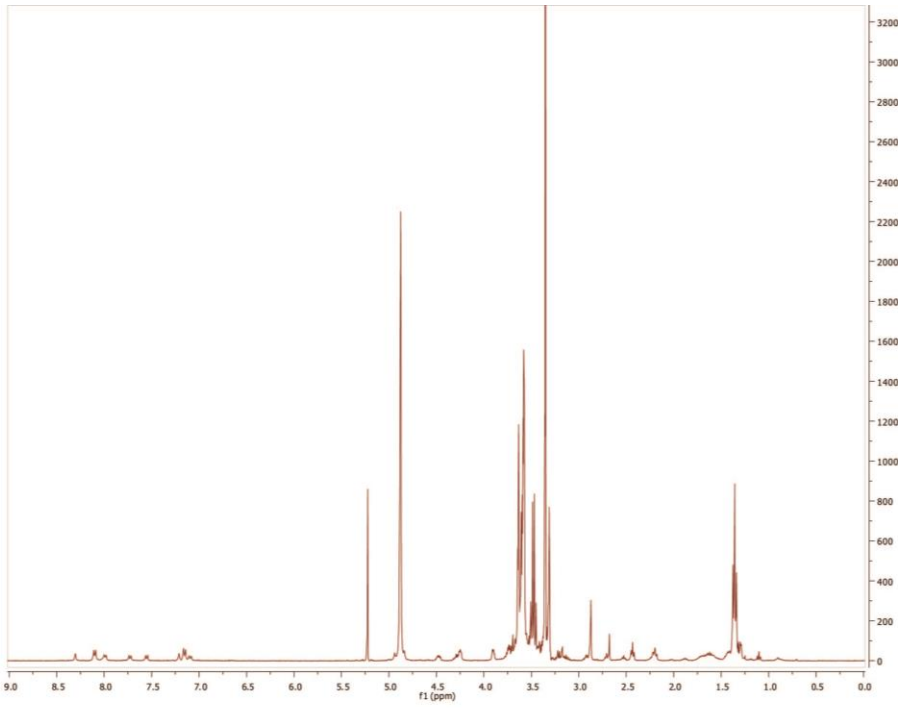
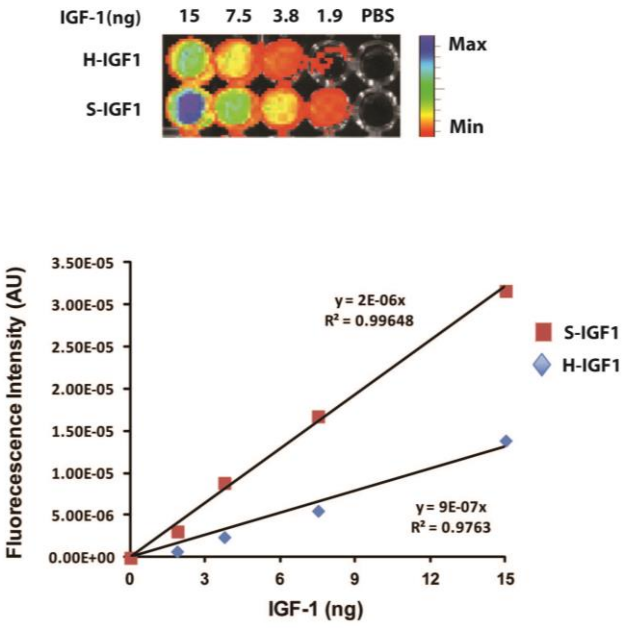
Supplemental Data

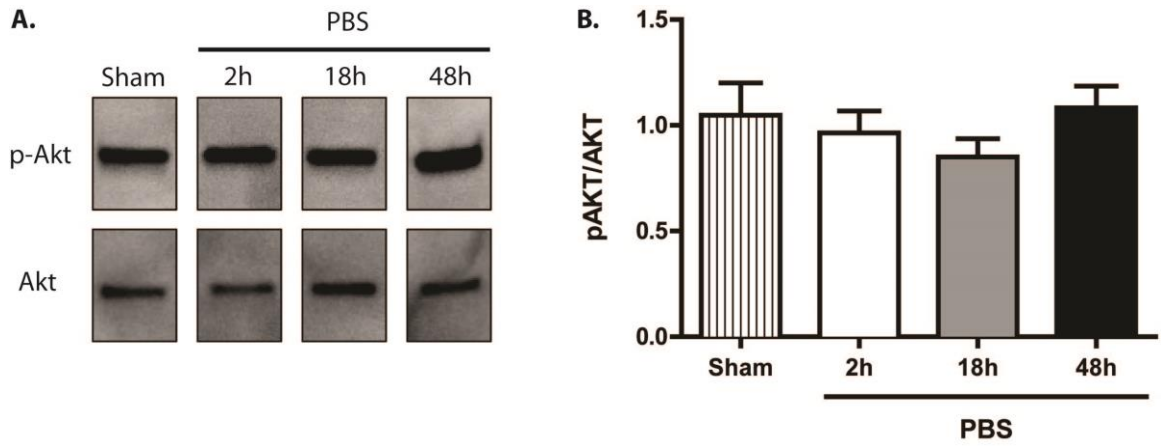
Supplemental Figure 1



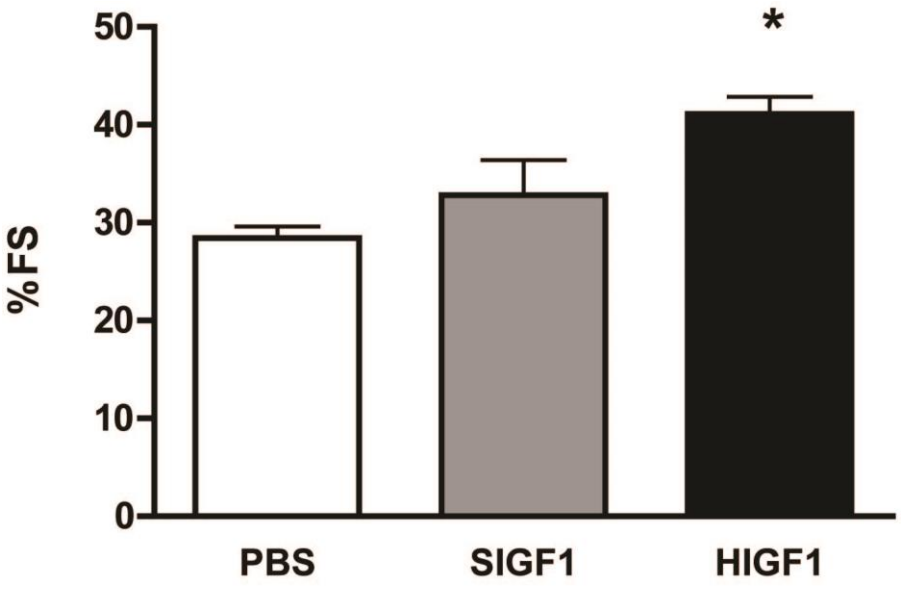
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure Legends

Figure 1: **Hoechst-Biotin Nuclear Magnetic Resonance (NMR) spectra.** Hoechst-biotin was characterized by ^{1}H-NMR (400 MHz, CD_3OD) by tracking aromatic Hoechst Protons (δ 8.10, d, 8.68 Hz, 2H; δ 7.99, d, 9.5 Hz, 2H; δ 7.73, d, 8.68 Hz, 1H; δ 7.55, d, 9.5 Hz, 1H; δ 7.15, m, 4H) to Biotin protons (δ 4.48, m, 1H; δ 4.30, m, 1H; δ 2.68, m, 1H; δ 2.20, t, 8.74 Hz, 2H).

Figure 2: **Hoechst-IGF-1 (H-IGF1) and Streptavidin-IGF-1 (S-IGF1) standard curves.** Differences in fluorescence efficiencies between H-IGF1 and S-IGF1 were accounted for by generating standard curves. Known amounts of H-IGF1 and S-IGF1, as measured by ELISA were added to a 96-well plate and imaged using a fluorescence-imaging camera (Xenogen IVIS 200 camera) with the same settings used to image harvested tissues. Fluorescence efficiency was measured and plotted against IGF-1 to generate standard curves for both H-IGF1 and S-IGF1. Compared to equal amounts of H-IGF1, S-IGF1 had considerably greater fluorescence efficiency.

Figure 3: **No changes in Akt phosphorylation between sham and PBS-treated mice following ischemia-reperfusion.** Proteins were extracted from animals subject to sham surgery or ischemia-reperfusion for the indicated time and Western analysis was performed for both phospho- and total Akt. Bands were quantified and expressed as a ratio of phospho-to-total (p-Akt/Akt). Representative blots and grouped data demonstrate no difference in Akt phosphorylation in any control group (n=4 per group).

Figure 4: **H-IGF1 preserves cardiac function two weeks following ischemia-reperfusion.** Echocardiography was used to measure cardiac contractility in PBS, S-IGF1 and H-IGF1 treated mice. Percent fractional shortening (%FS), an index of systolic function, was significantly greater in H-IGF1 treated mice compared to S-IGF1 and PBS treated mice (n=7-11 per group, *, $p<0.05$ vs. S-IGF1, one-way ANOVA followed by Tukey's post-test).