FIGURE LEGENDS

Supplemental Figure 1. A: Dose-dependent decrease in DHR oxidation rate (as measured by changes in fluorescence per minute) by incubation with increasing concentration of HDL (hatched columns), or LDL (solid black columns) that were isolated by FPLC from a healthy donor and stable amount of DHR (50 uM). When increasing concentrations of both LDL and HDL (hatched grey columns) were coincubated with DHR (50 uM) at a ratio of 1:1 (2.5 ug each) there was increased fluorescence quenching and the variability (as demonstrated by error bars) increased significantly. The values represent means ± SD of quadruplicate samples.

B: The correlation between DHR oxidation rate and the added amount of HDL (triangles) or LDL that were isolated by FPLC from healthy donor in stable concentration of DHR (50 uM) is shown. Although the correlation remains similar for HDL and LDL independently and for their combination (1:1 ratio, 2.5 ug each), the variability increased significantly (increased error bars)

Supplemental Figure 2. Oxidation of DHR in the presence of six different samples of HDL [3 from patients with coronary artery disease (CAD) and 3 from healthy volunteers (Non CAD)] was assessed as described in Figure 1, using 2.5μg (cholesterol) of added HDL. The data (means of quadruplicates) from three independent experiments are plotted. The experiments were repeated after DHR (hatched columns) was almost fully oxidized (air oxidation at room temperature for 6 hours). There was a ~30-fold increase in the slope of oxidation of DHR after 6 hours of air oxidation. The oxidized DHR (DHR ox) was diluted 30-fold to give comparable fluorescence to the baseline DHR and experiments after addition of HDL were

repeated. There was a significant increase in the variability of oxidation of DHR ox compared to baseline DHR and addition of HDL did not cause significant fluorescence quenching. These results indicate that most of DHR had converted into R and did not react with HDL confirming the fact that fluorescence quenching is the result of DHR-HDL interaction.

Supplemental Figure 3. Dose-dependent decrease in DHR oxidation rate (as measured by changes in fluorescence per minute) by incubation of 50 uM of DHR with increasing concentration of HDL (2.5, 5, 10, 20, 40 ug) (hatched columns), or LDL (solid black columns) that were isolated by ultracentrifugation from a healthy donor and stable amount of DHR (50 uM). Fluorescence intensity was determined after 1 h of incubation at 37° C. The values represent means ± SD of quadruplicate samples.

Supplemental Figure 4. HDL produces a concentration-dependent decline in the amount of DHR, as determined by LC/MS/MS. HDL was isolated from a healthy donor by sequential ultracentrifugation, and 0-10 ug cholesterol were combined with DHR in triplicate, in the manner of the fluorometric assay (50 uM DHR in a total volume of 175 uL in HEPES-buffered saline). After the incubation, urate was added (0.025 mM final concentration) to slow the further oxidation of DHR. The amount of DHR remaining in each sample was then determined by LC/MS/MS. There was a HDL-concentration dependent decline in the amount of DHR present in the samples. The amount of DHR remaining in the 10 ug HDL sample was significantly lower than the 0, 0.625, and 1.25 ug samples (*, $p < 0.005$, n=3).

Supplemental Figure 5. The effect of purified, commercially available, lipid-free apolipoprotein AI (ApoAI) from a healthy donor on DHR oxidation rate (as measured by changes in fluorescence per minute) was assessed by incubation of 50 uM of DHR with increasing concentration of ApoAI (0.6, 1.3, 2.5, 5.0, 10.0 mg/dl). The normal blood levels of ApoAI range from 90-130 mg/dl which correspond to normal blood levels of HDL cholesterol that ranges from 30-50 mg/dl (~3:1 ratio) (1;21). The concentration of HDL used in the 96 well DHR assay ranges from 1.25 ug-5 ug/0.175 ml or 0.7-2.9 mg/dl which corresponds to an ApoAI range of ~2-9 mg/dl. Fluorescence intensity was determined after 1 h of incubation at 37° C. The values represent means ± SD of quadruplicate samples. There was no concentration dependent effect of apoAI (at concentration > 2 mg/dl) on DHR oxidation rate.

Supplemental Figure 6. Influence of azide, phosphate-buffered saline, and pH on measurements of HDL oxidative activity. Measurements of HDL influence on DHR oxidation were assessed using 50µM DHR exposed to 5µg (cholesterol) of FPLC-purified HDL, in 175µl of varying diluents. Rates of oxidation for different concentrations of added HDL are plotted as in Figure 2, as means of quadruplicates. A. Results are plotted for: saline with 150mM NaCl and HEPES 20mM pH 7.4, versus saline with 150mM NaCl and HEPES 20mM pH 7.4 with sodium azide 3mM concentration (concentration that was used in the buffer of the previously described cell free assay), versus standard phosphate-buffered saline (PBS), versus PBS with sodium azide 3mM concentration. B. Results are plotted for saline with 150mM NaCl with HEPES 20mM at varying pH levels.

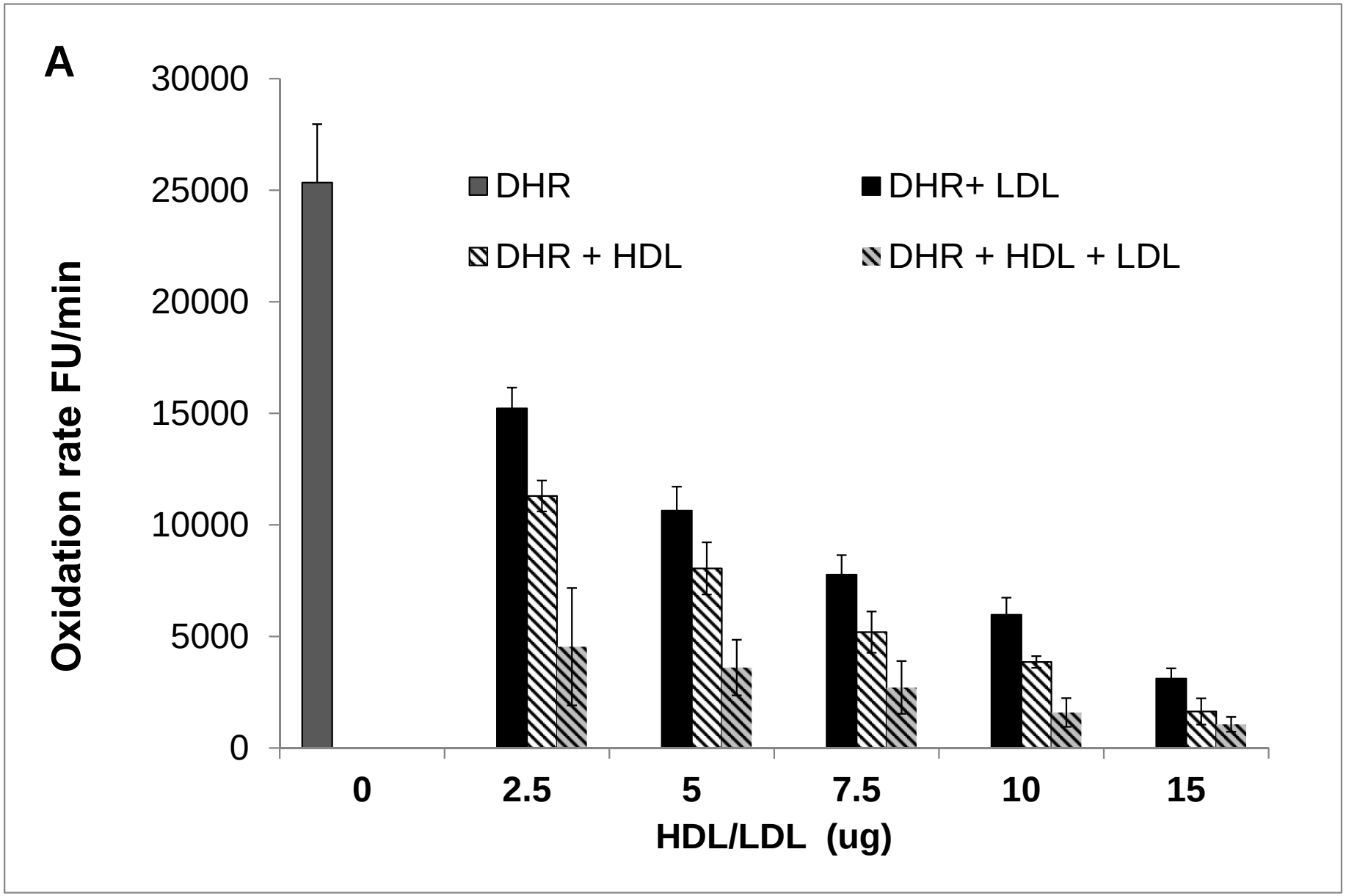
Supplemental Figure 7. Influence of method of HDL isolation on measurements of HDL oxidative activity. A. Measurements of HDL influence on DHR oxidation were assessed using 100 µl in a 384 well flat bottom plate and the rate of change in fluorescence was measured as in Figure 1. 15 µM DHR was exposed to 2.5µg (cholesterol) of FPLC-purified (FPLC) or ultracentrifugation-purified HDL (UC) that was isolated from 10 patients with coronary artery disease (CAD) and 10 healthy volunteers (Non CAD) as described in Materials and Methods Rates of oxidation of DHR are plotted as means of quadruplicates for each sample. B. Rates of oxidation of DHR are plotted as means of 10 patients with coronary artery disease (CAD) and 10 healthy volunteers (Non CAD) using ultracentrifugation (UC) or FPLC for isolation of HDL. The oxidation rate of DHR was significantly higher when HDL isolated by ultracentrifugation was added compared to when HDL isolated by FPLC was added ($p< 0.001$, $n=20$). *** $p<0.001$

Supplemental Figure 8. Correlation of effect of HDL on DHR oxidation using different methods of HDL isolation. Ten HDL samples from 10 healthy volunteers (Non CAD) isolated by both FPLC and ultracentrifugation were assessed for their ability to inhibit DHR oxidation as shown in Figure 4. The values from each method of HDL isolation are plotted against each other. A. HDL isolation using dextran sulfate versus HDL isolation using FPLC B. HDL isolation using dextran sulfate versus HDL isolation using ultracentrifugation (UC) C. HDL isolation using dextran sulfate versus HDL isolation using polyethylene glycol (PEG) D. HDL isolation using polyethylene glycol (PEG) versus HDL isolation using FPLC E. HDL isolation using polyethylene glycol (PEG) versus HDL isolation using ultracentrifugation (UC) F. HDL isolation using FPLC versus HDL isolation using ultracentrifugation (UC).

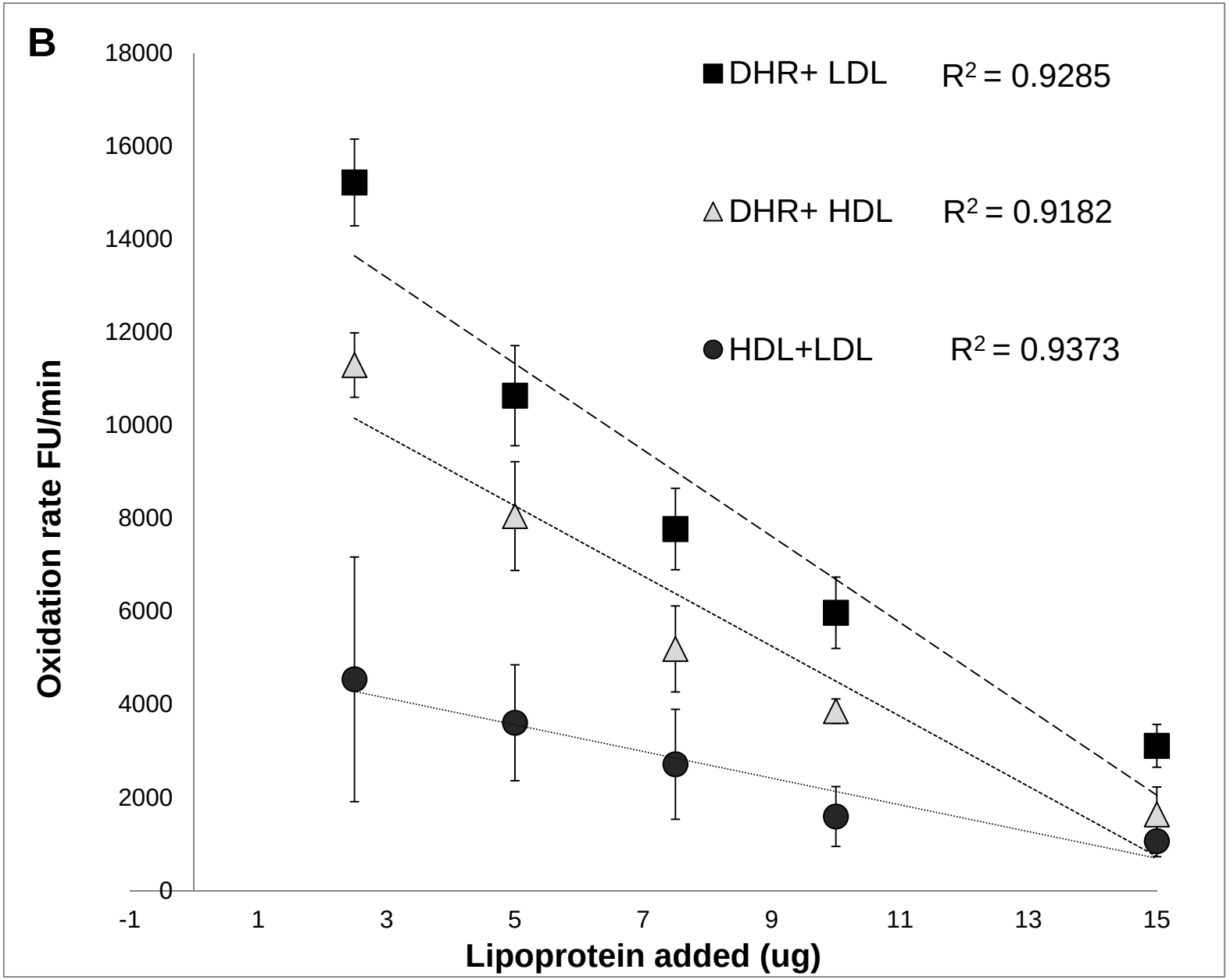
Supplemental Figure 9. The DHR assay can detect dysfunctional HDL in patients with HIV infection. HDL was isolated from plasma of 4 patients with Human Immunodeficiency Virus Infection (HIV) who were not treated with antiretroviral therapy and 4 healthy volunteers (non-HIV) using polyethylene glycol (PEG). The HDL from each patient with HIV (pHDL) was mixed with HDL from non-HIV patient at certain ratios so that the % of pHDL in each reaction was 0, 20, 40, 60, 80, 100% and the total amount of added cholesterol in each reaction was stable (2.5 ug of cholesterol). Oxidation of DHR in the presence of four different pairs of HDL (Group A, B, C, D) was assessed as described in Figure 1, using 2.5μg (cholesterol) of added HDL. The correlation coefficient (R^2) of the DHR oxidation slope with the % of pHDL in each reaction is shown for each group. The statistically significant correlation in all four pairs ($R^2 > 0.8$, $p < 0.01$) demonstrates the ability of the DHR assay to detect pHDL in HIV infection even when the HDL from these patients was mixed with HDL from non-HIV patients at various ratios.

Supplemental Figure 10. Correlation of results in the 96 well format to results in a 384 well format. Thirteen samples of FPLC-purified HDL were assessed for oxidative effects on DHR using both the 96 well plate format and the 384 well format. The values from each method are plotted against each other.

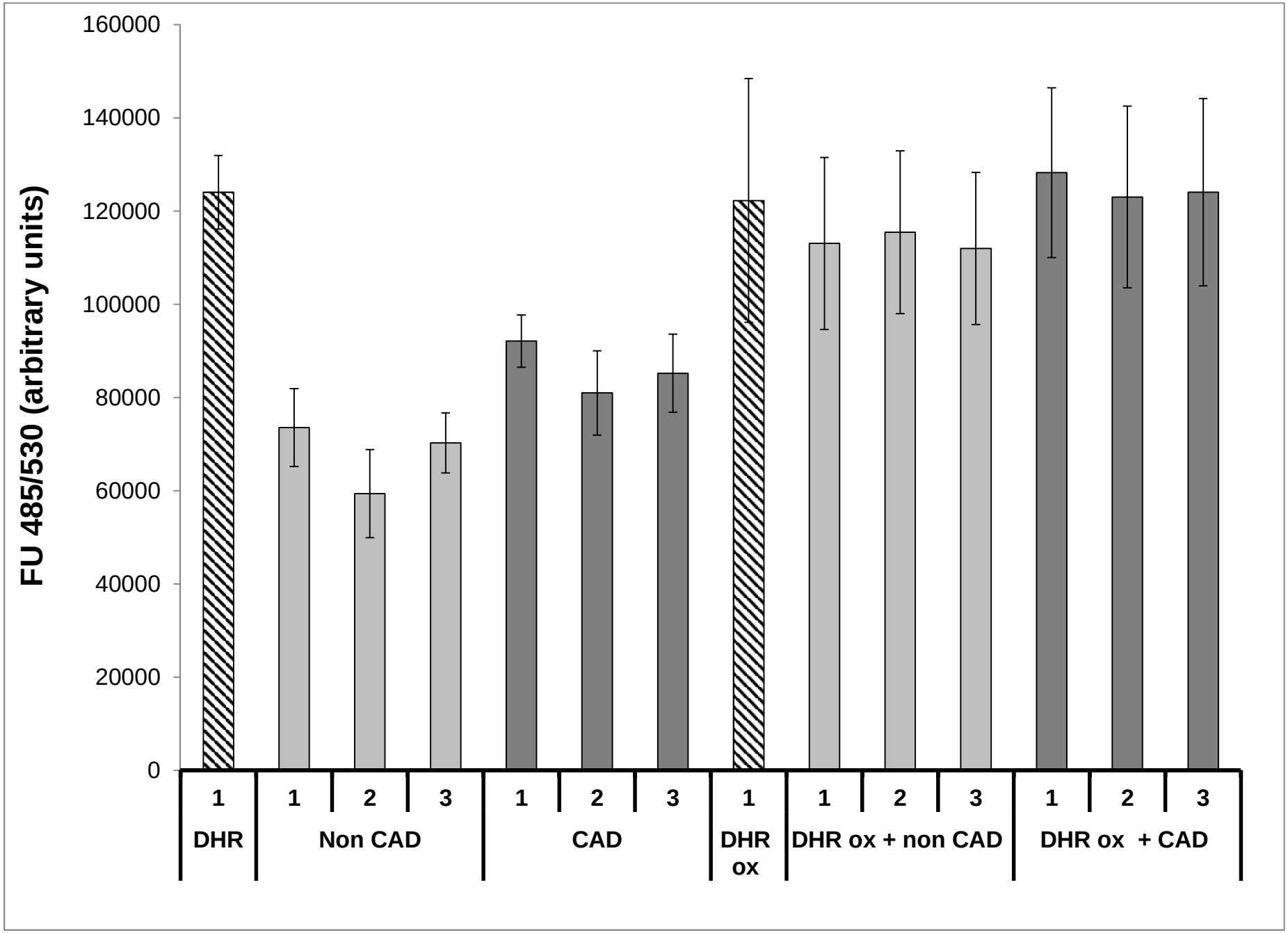
Supplemental Figure 1A



Supplemental Figure 1B



Supplemental Figure 2



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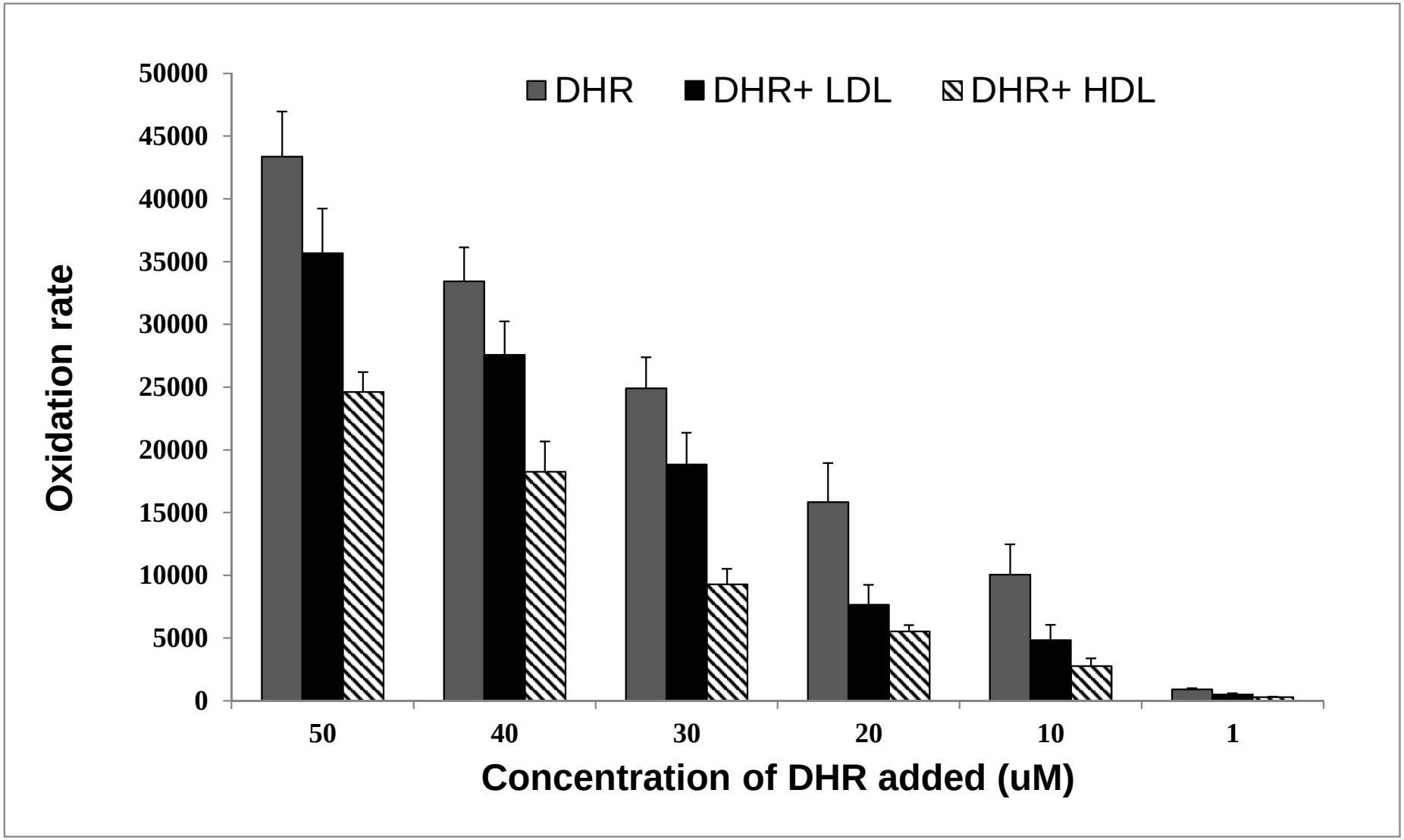

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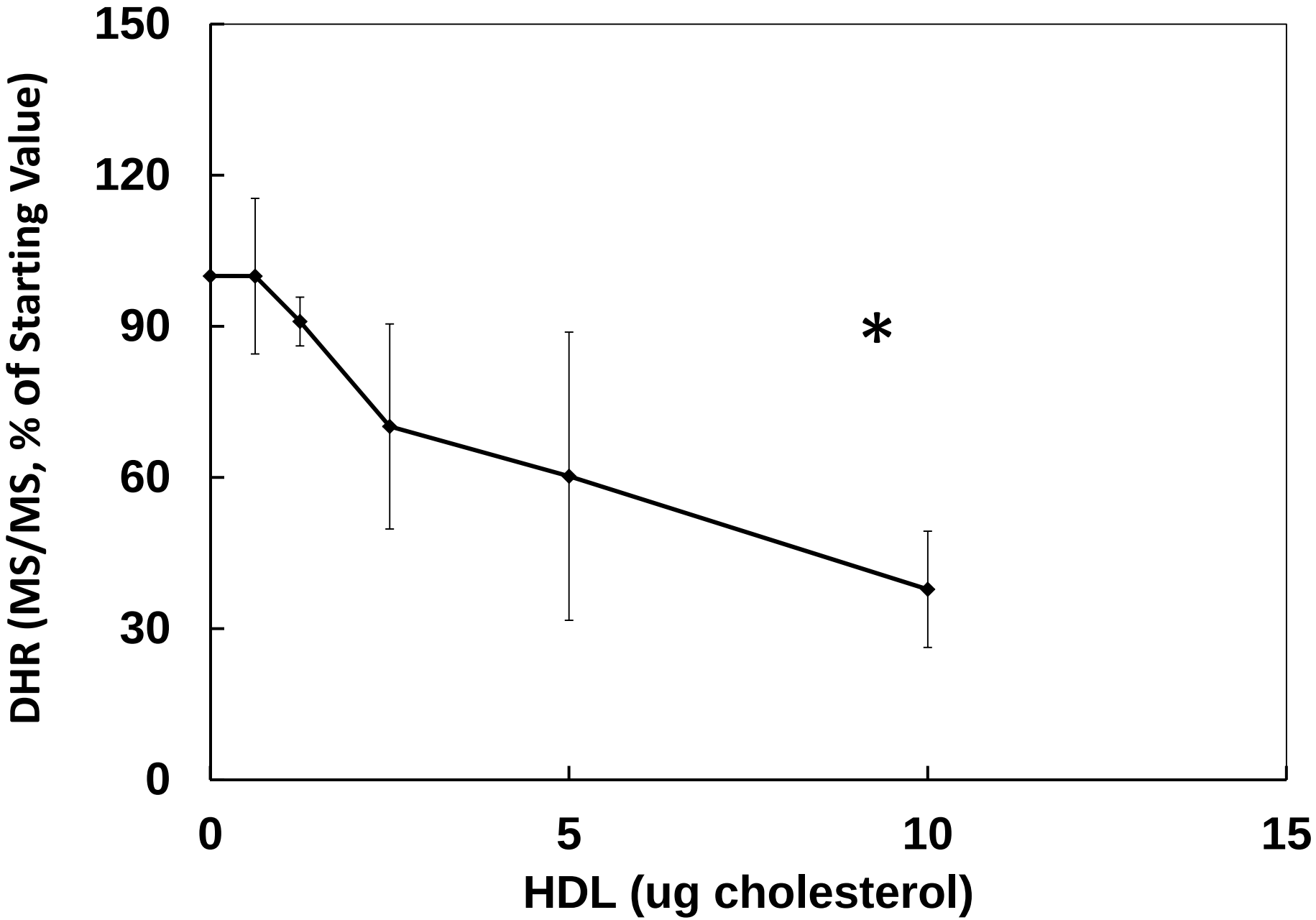
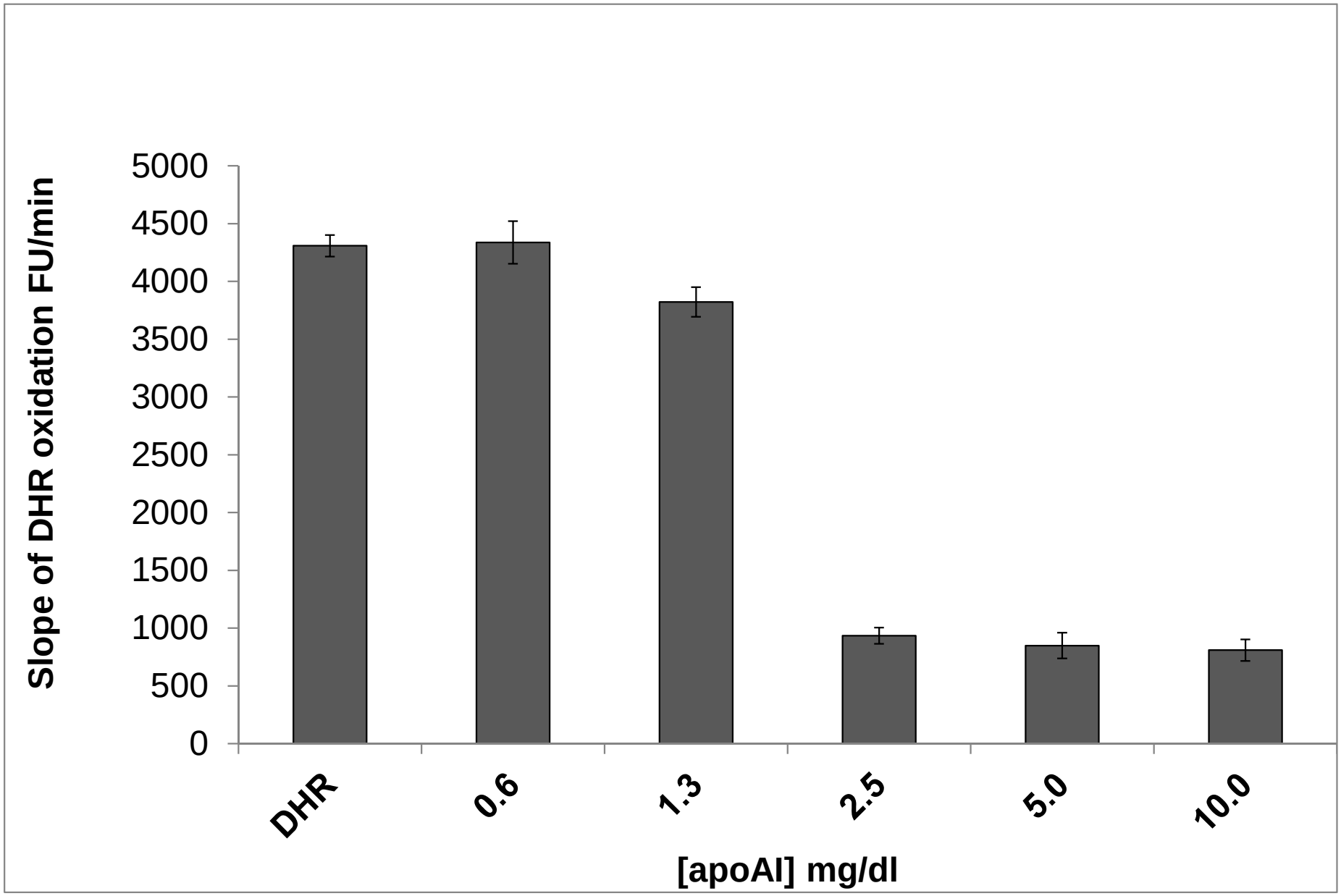
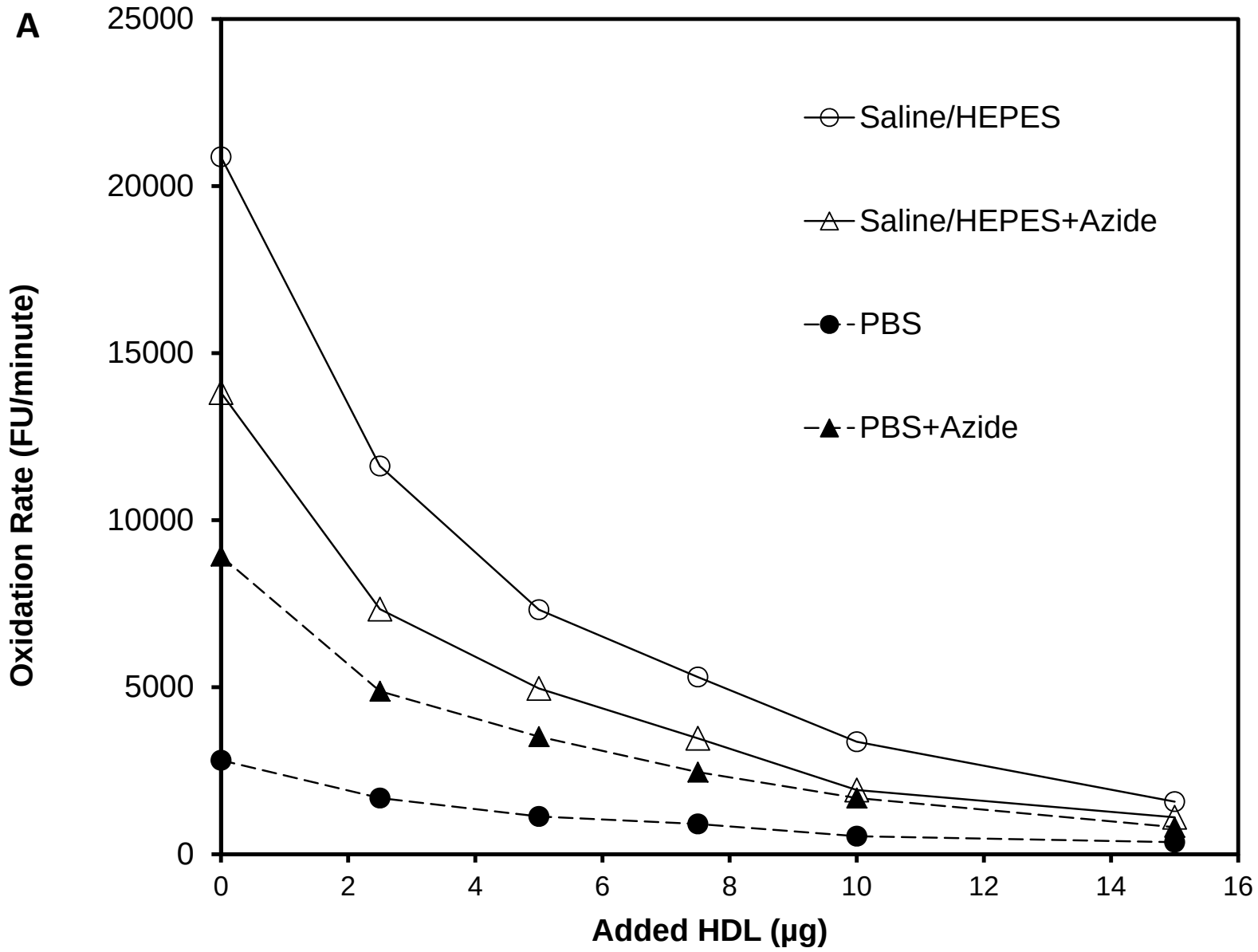


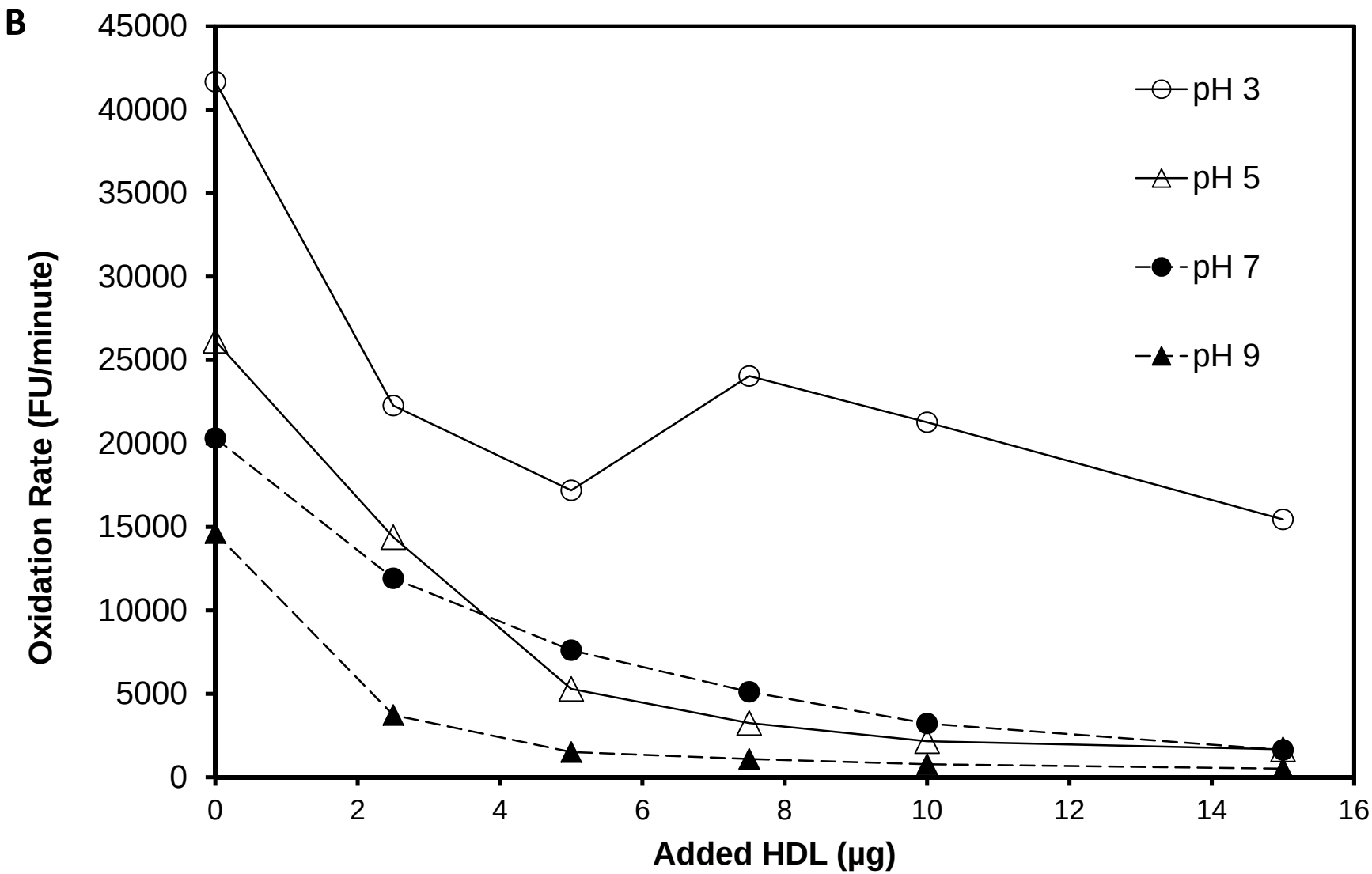
Supplemental Figure 5



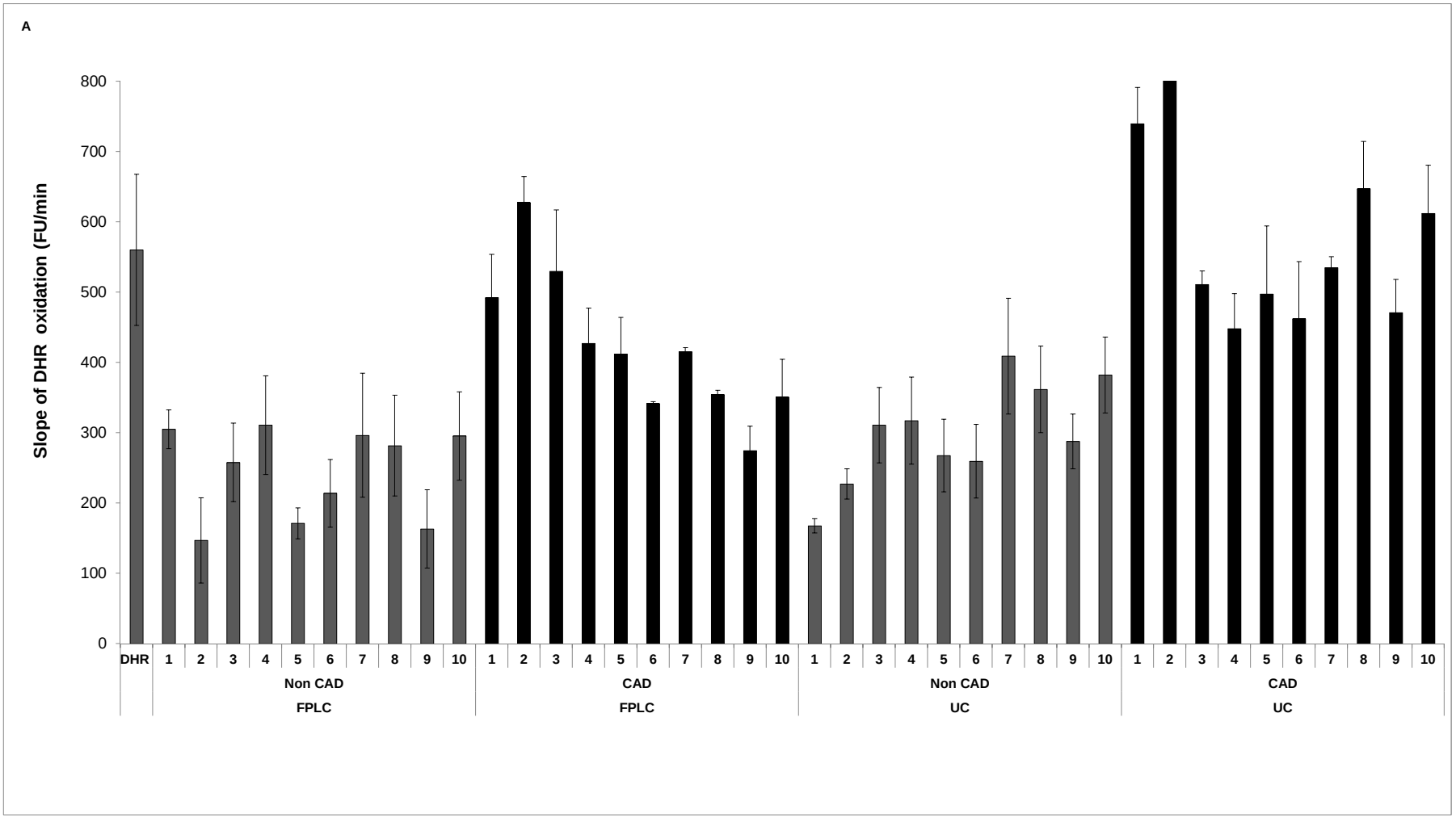
Supplemental Figure 6A



Supplemental Figure 6B



Supplemental Figure 7A



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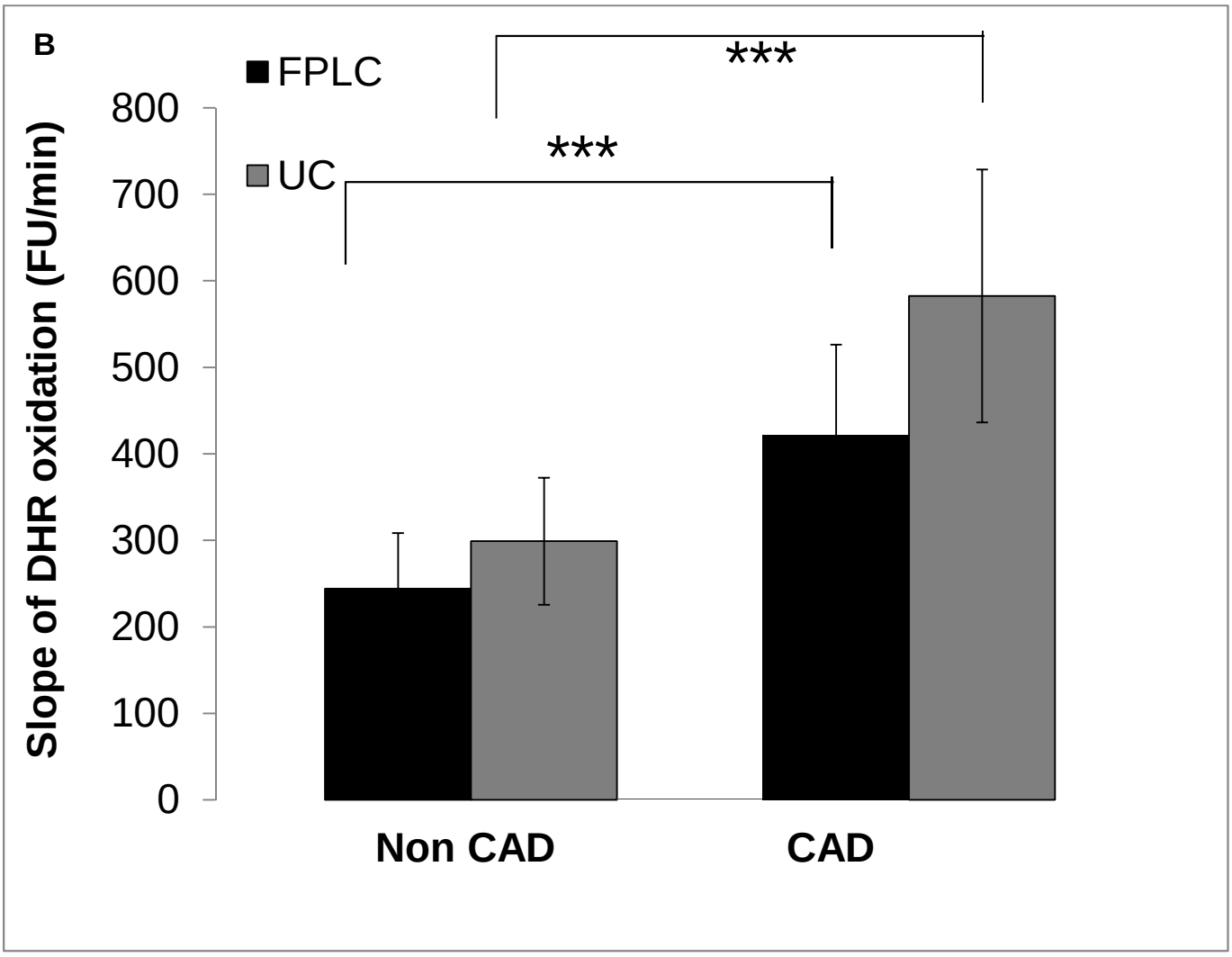
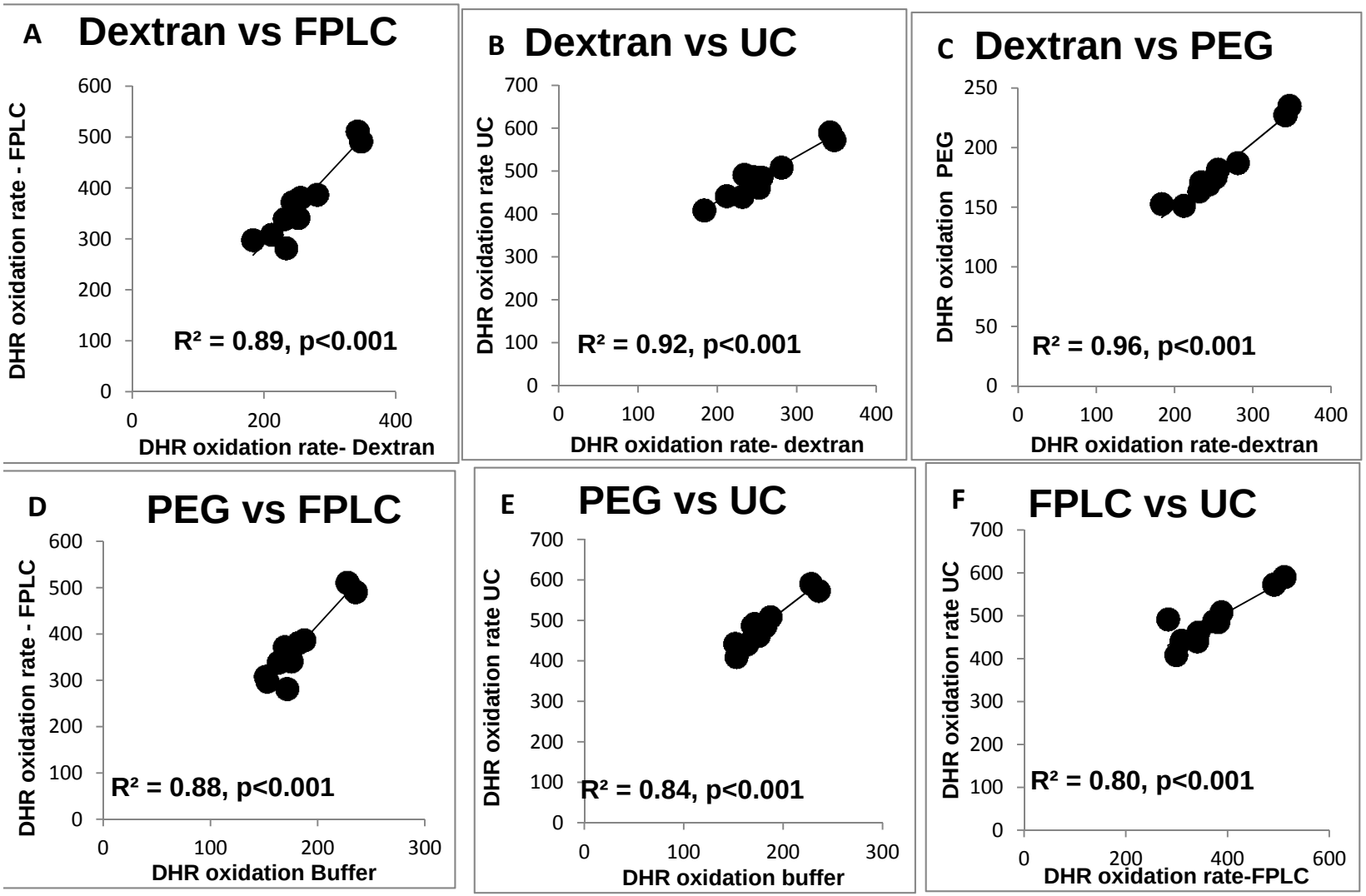
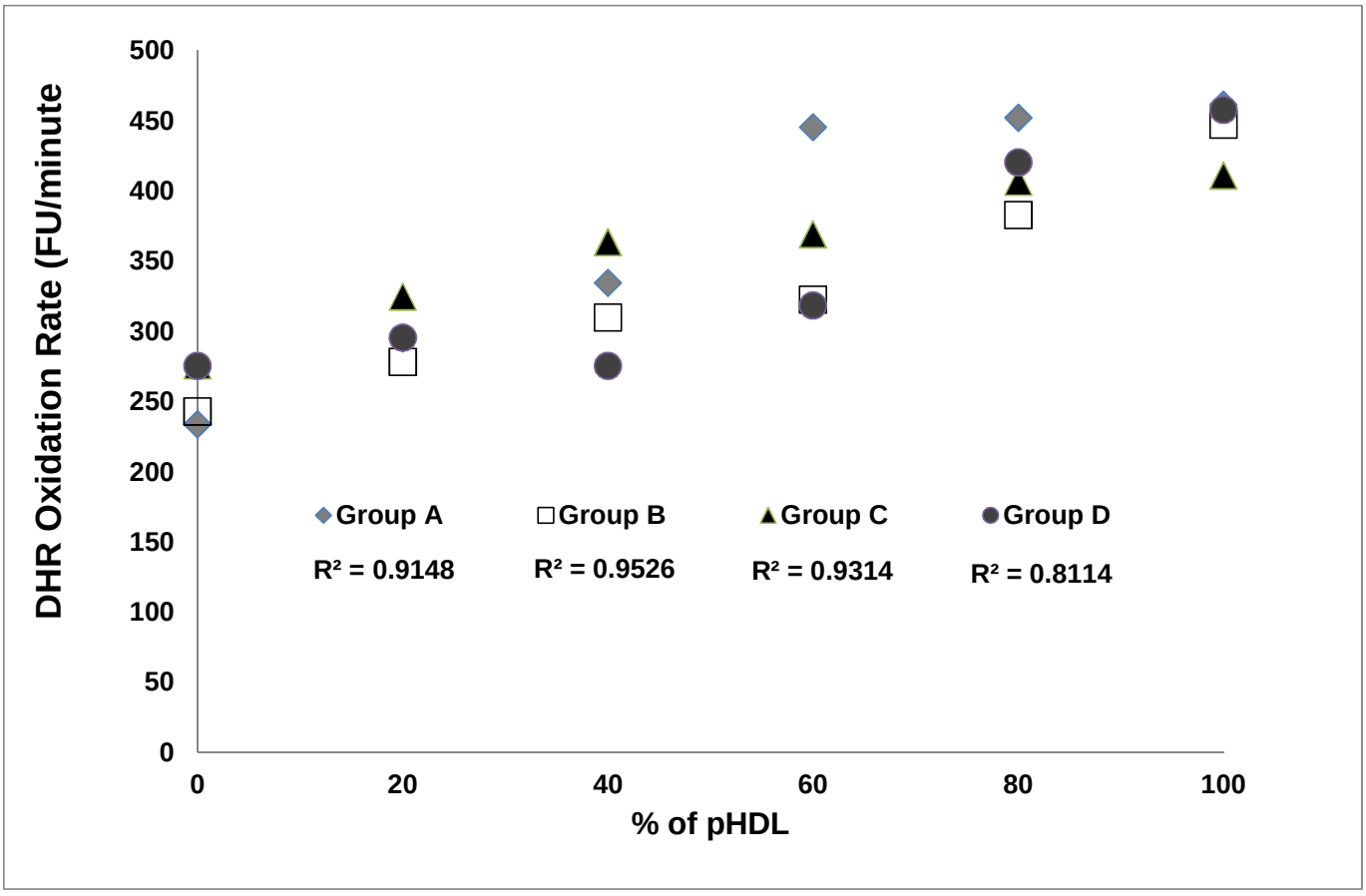


Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10