Supplementary Material
for

Iron Prochelator BSIH Protects Retinal Pigment Epithelial Cells against Cell Death Induced by Hydrogen Peroxide

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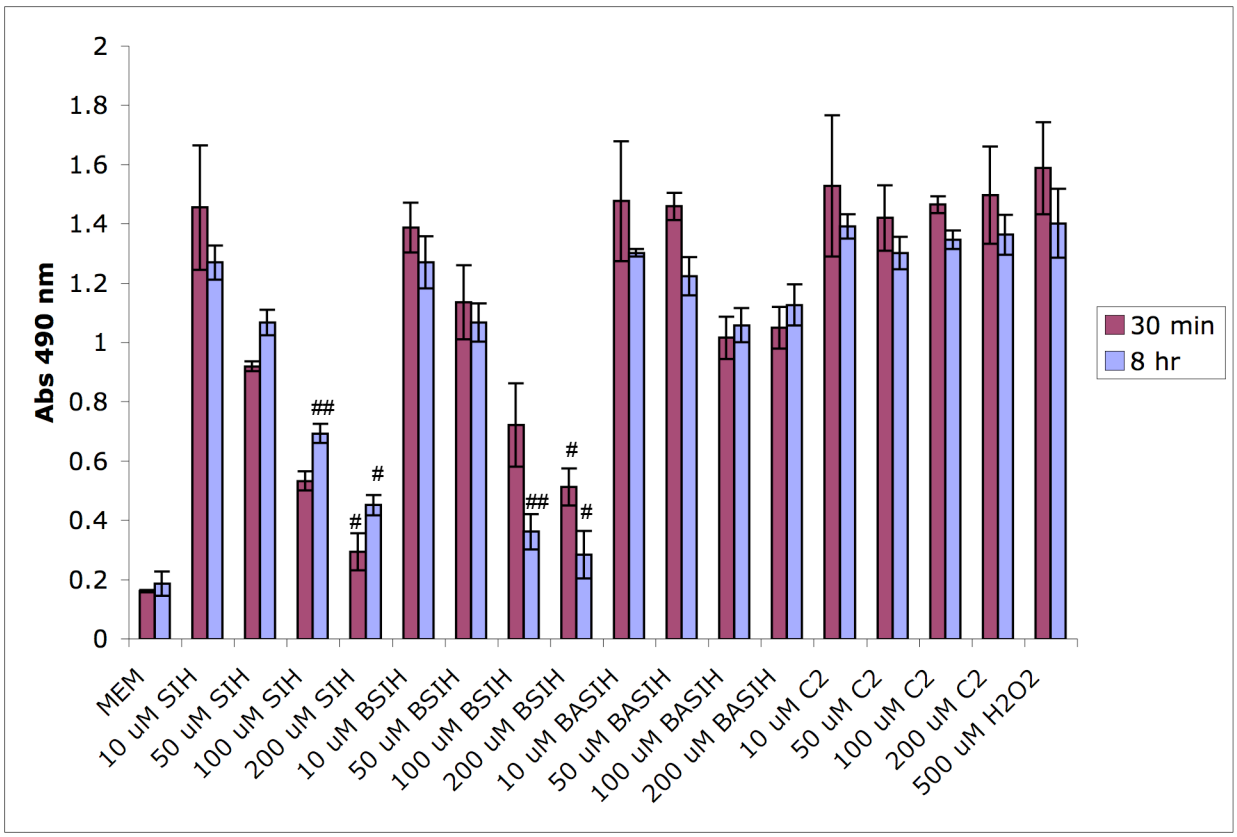

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Figure S1. LDH assay of ARPE-19 cells pretreated with chelator or prochelator for 30 min or 8 h then with 500 μM H_2O_2 for 18 h. The absorbance at 490 indicates release of LDH, signifying cell death. "MEM" is a control of cell viability that received neither pre-treatment nor H_2O_2. "H2O2" is a control showing maximal cell death when cells are treated with 500 μM H_2O_2 without any pre-treatment. SIH, BSIH, BASIH are the chelator and prochelators shown in Figure 1; C2 is an analog of BSIH in which the boronate mask is positioned to release a non-iron-binding product. Statistical significance is shown for comparison between SIH and BSIH at the same concentration and pretreatment time, # $p<0.05$, ## $p<0.01$.

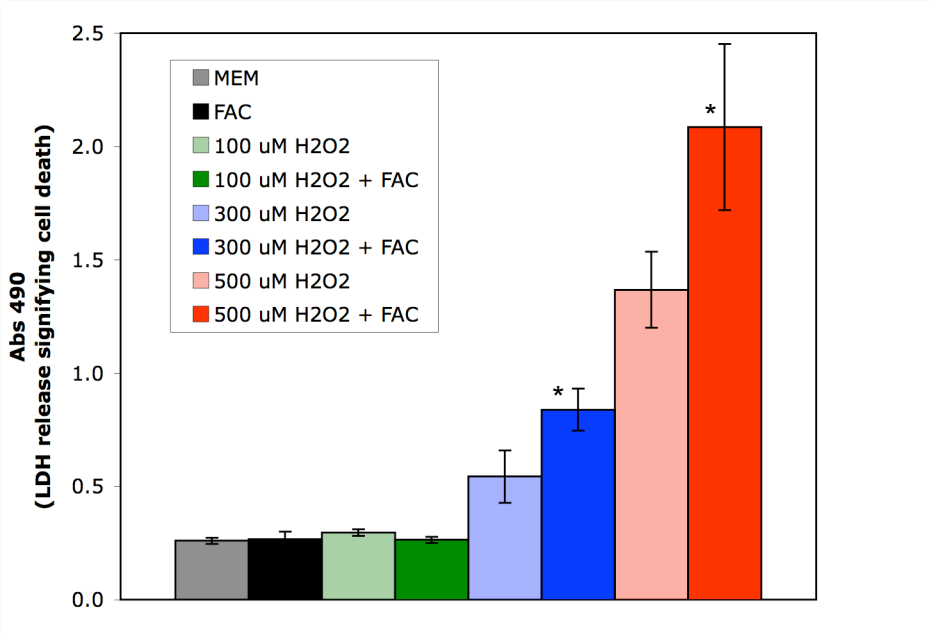

Figure S2. LDH assay of ARPE-19 cells subjected to 100, 300, or 500 μM H_2O_2 with or without pretreatment with 250 μM ferric ammonium citrate (FAC) for 24 h. Preloading the cells with iron makes them more susceptible to H_2O_2-induced cell death, as shown by the greater LDH release for cells that received both FAC and H_2O_2 compared to cells that were exposed to the equiv amount of H_2O_2 without FAC pretreatment. Cell death was monitored by the A_{490} of released LDH 18 h after peroxide exposure. "MEM" is a control that was not exposed to FAC or H_2O_2; "FAC" is a control that received only FAC but no H_2O_2. Statistical significance is shown relative to samples that received the same concentration of H_2O_2 but no FAC, * $p<0.05$.

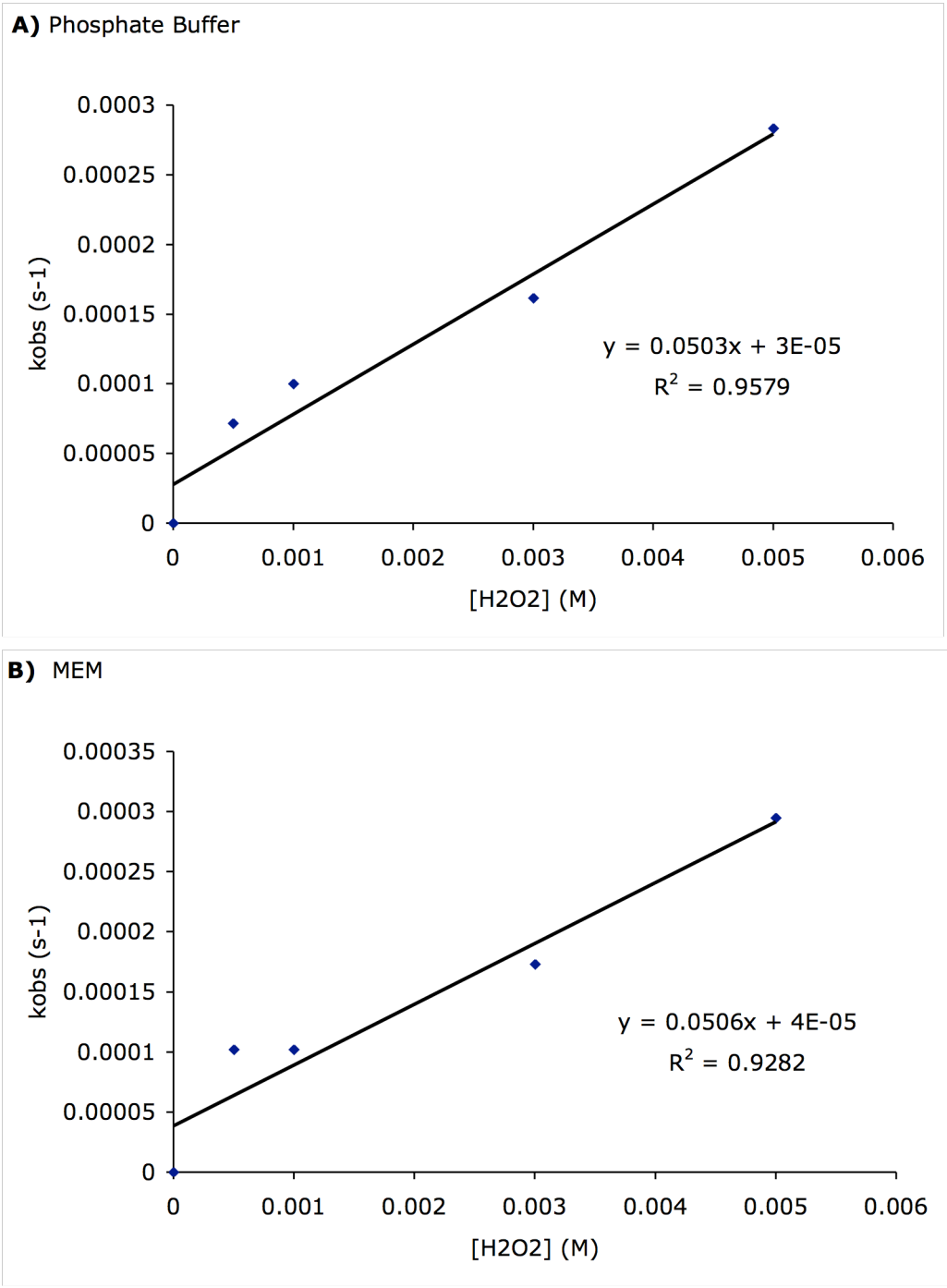

Figure S3. Plots of k_{obs} vs H_2O_2 concentration for the conversion of 50 μM BSIH to SIH in **A)** 20 mM $NaHPO_4$ buffer at pH 7.4 or **B)** MEM cell culture media at pH 7.5. Spectral changes were monitored by UV-vis spectrophotometry to get the observed pseudo-first-order rate constants, k_{obs}. A rate constant k = 0.05 $M^{-1}s^{-1}$ was obtained from the slope of these lines for both solution conditions. The same rate was observed previously in a 50/50 methanol/phosphate buffer solution.[1]

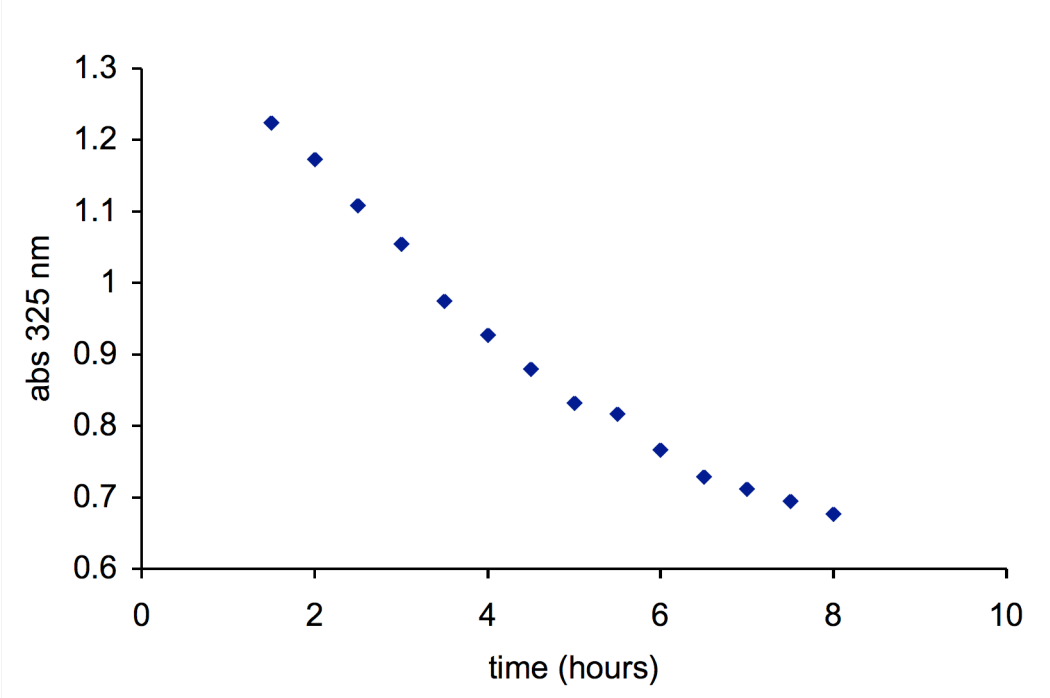

Figure S4. Degradation of SIH in MEM at 37 °C. The absorbance of SIH at 325 nm was recorded every 30 minutes for 8 h for a solution of 100 μM SIH in MEM incubated at 37° C. The half-life for SIH stability under these conditions is $t_{1/2}$ = 7 h. A previous report found $t_{1/2}$ = 2.7 h for SIH in RPMI cell culture media.[2]

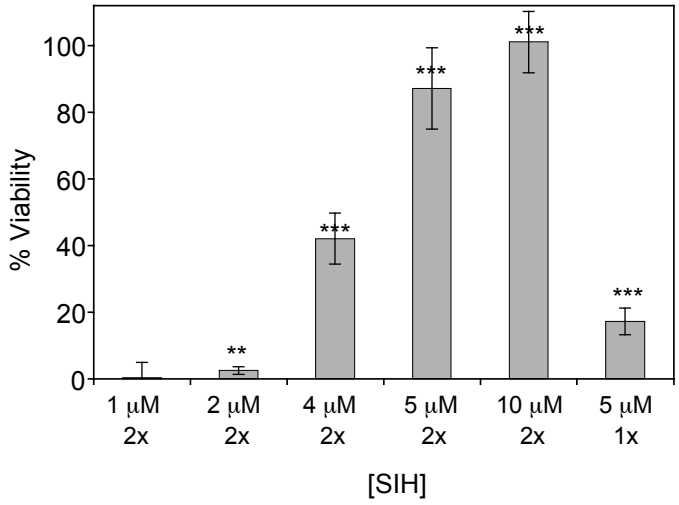

Figure S5. Double dosing protocol improves the efficacy of SIH. ARPE-19 cells were pre-incubated with 1, 2, 4, 5, or 10 μM SIH for 30 min, followed by treatment with 500 μM H_2O_2 and an additional dose of SIH at the same concentration as the first dose. The final column shows data for cells that were not pre-treated, but only received 5 μM SIH at the same time they were treated with 500 μM H_2O_2. Cell viability was monitored by the CellTiter Blue assay after 15 h. Statistical significance is indicated relative to samples exposed to H_2O_2 but given no chelator/prochelator treatment: ** $p<0.01$, *** $p<0.001$.

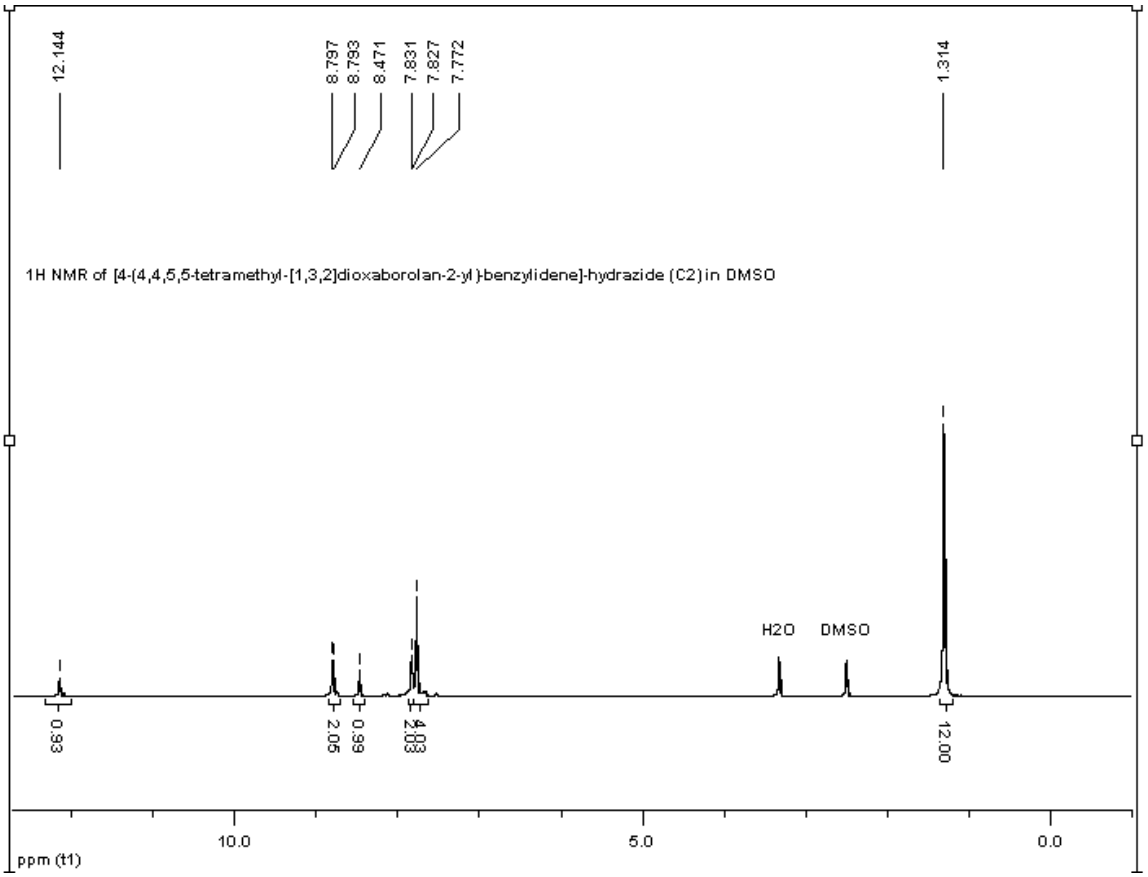

Figure S6. ^{1}H NMR spectrum of C2 ([4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzylidene]-hydrazide).

References.

[1] L. K. Charkoudian, D. M. Pham, A. Kwan, A. Vangeloff, K. J. Franz, Dalton Trans., 43 (2007) 4873-5092.

[2] J. L. Buss, P. Ponka, Biochim. Biophys. Acta, 1619 (2003) 177-186.