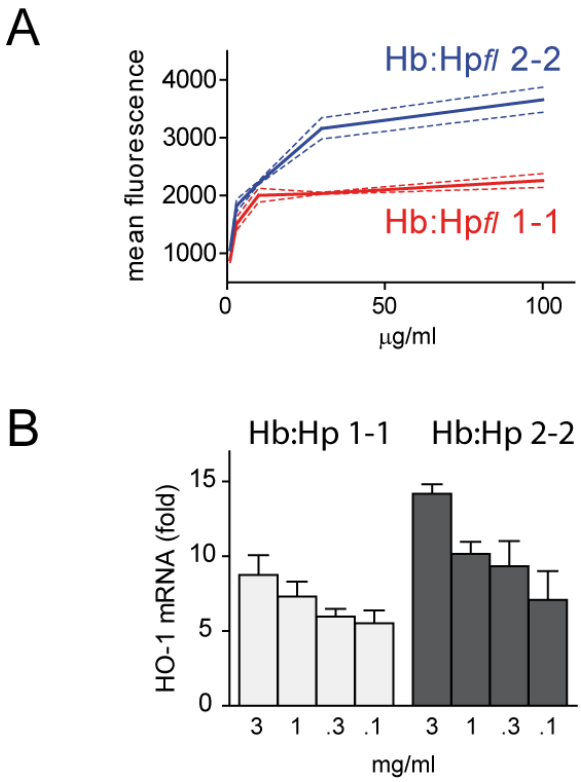
SUPPLEMENTARY FIGURE 5



Cellular complex uptake and HO-1 imRNA induction by Hb:Hp 1-1 and Hb:Hp 2-2

(A) CD163^{+} HEK293 cells were incubated for 30 minutes with Alexa-488-labeled Hb:Hp$_{fl}$ 1-1 or Hb:Hp$_{fl}$ 2-2 at five different concentrations: 100 ug/ml, 30 ug/ml, 10 ug/ml, 3 ug/ml, and 1 ug/ml. After washing, mean cellular fluorescence was determined by FACS. Data is represented as mean ± SEM of triplicate experiments. **(B)** CD163+ HEK293 cells were incubated for eight hours with HPLC purified HbHp1-1 or HbHp2-2 complexes. Each ligand was applied in four different concentrations (3 mg/ml, 1 mg/ml, and 0.3 mg/ml, 0.1 mg/ml). HO-1 Induction was determined as fold induction relative to non-treated cells by quantitative Real-Time PCR and normalized to GAPDH. The results are shown as mean ± SEM of triplicate experiments.

Methods: CD163-mediated endocytosis of fluorescent Hb:Hp complexes was studied using CD163 transduced HEK293 cells, as described [1]. Hp 1-1 and 2-2, respectively, were labeled with Alexa Fluor-488 dye using the Alexa-488 labeling kit (Molecular Probes, Invitrogen) according to the manufacturer's protocol. Equal labeling of Hp was confirmed with a fluorescence spectrophotometer. To quantify HbHp$_{fl}$*488* 1-1/2-2 uptake, complexes were incubated on CD163-expressing HEK293 cells for 45 minutes at 37 °C. HO-1 induction in CD163-expressing HEK293 cells was measured by RT-PCR as described previously [2].

1. Schaer DJ, Schaer CA, Buehler PW, et al. CD163 is the macrophage scavenger receptor for native and chemically modified hemoglobins in the absence of haptoglobin. *Blood*. 2006;107(1):373-380.

2. Schaer CA, Schoedon G, Imhof A, Kurrer MO, Schaer DJ. Constitutive endocytosis of CD163 mediates hemoglobin-heme uptake and determines the noninflammatory and protective transcriptional response of macrophages to hemoglobin. *Circ Res*. 2006;99(9):943-950.