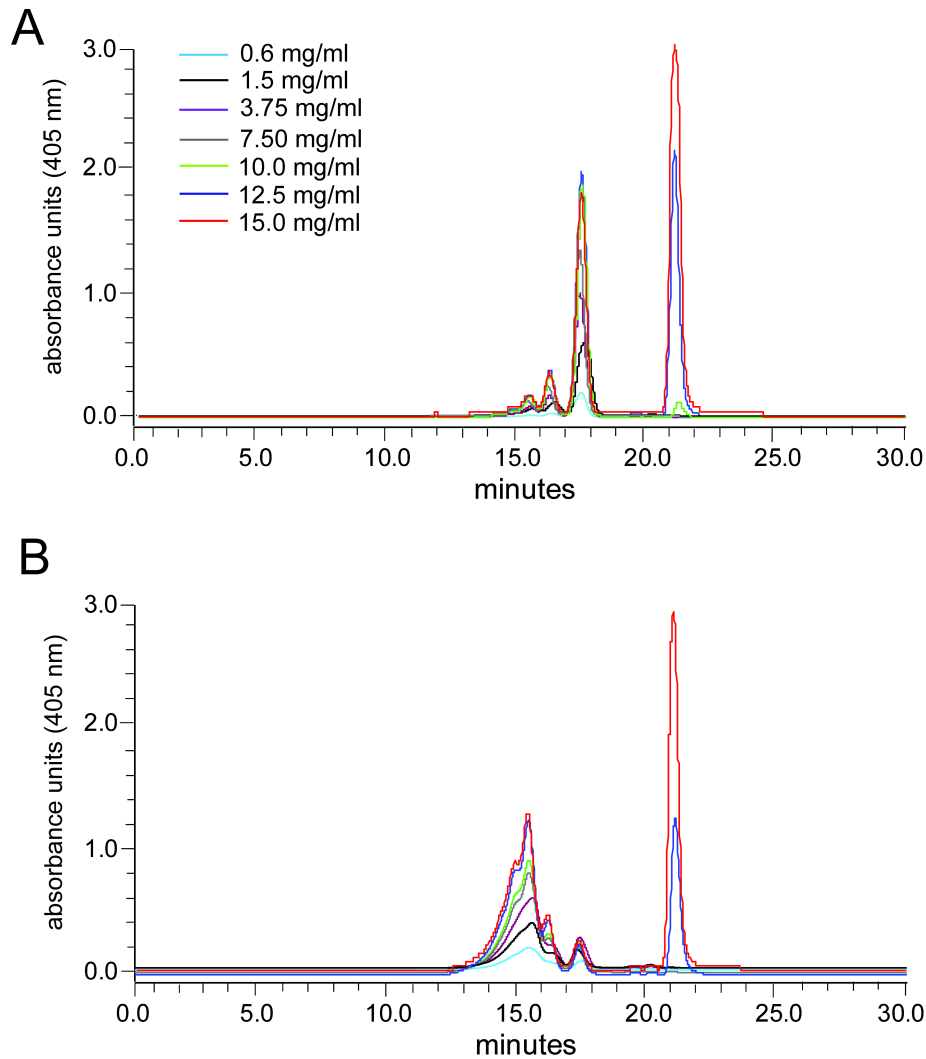


SUPPLEMENTARY FIGURE 4

**Hb binding of guinea pig plasma after infusion of Hp 1-1 and 2-2**

(A) Demonstrates representative serial chromatography of Hb binding to Hp 1-1 in guinea pigs plasma collected after a 200 mg dose. Saturation of Hp1-1 occurred at approximately 10 mg/ml. **(B)** Demonstrates representative serial chromatography of Hb binding to Hp 2-2 in guinea pigs plasma collected after a 200 mg dose. Saturation of Hp 2-2 occurred at approximately 10 mg/ml. The Hb-Hp data are shown in Figure 6C of the main paper.

Methods: Guinea pigs (n=2/group) were dosed with a bolus top-load of 240 mg (4 ml) of either Hp 1-1 or Hp 2-2. Immediately after bolus infusions animals were anesthetized with ketamine (40mg/kg)/xylazine (5mg/kg) and exsanguinated. Plasma was obtained by centrifugation (6000 rpm x 20 minutes). Plasma aliquots of 250 μ M were pipetted into eppendorf tubes and Hb stock solution 150 mg/ml was added to each stock solution to obtain the following Hb concentrations: 0.6, 1.5, 3.75, 7.5, 10, 12.5 and 15.0 mg/ml Hb. All samples were run on a Waters 2535 quaternary gradient HPLC system with a Waters 2998 photodiode array detector. The system was attached to Biosep-SEC 3000 (7.5 x 6000 mm) size exclusion chromatography column with a 50 mM phosphate buffer running buffer, pH 7.4 at flow rate of 1.0 ml/min. Samples were monitored at 280 and 405 nm. All chromatographs were overlaid to show the extent of Hb binding to Hp 1-1 and 2-2. Areas (AU*min) of Hb bound Hp components (14-17 minutes) were plotted against Hb concentration to determine the amount of Hp and extent of binding in plasma.