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Supplementary Materials for

Circulating hemopexin modulates anthracycline cardiac toxicity in patients and in mice

Jing Liu *et al.*

Corresponding author: Aarti Asnani, aasnani@bidmc.harvard.edu

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The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

Supplemental Data

Supplementary Figures

Circulating hemopexin modulates anthracycline cardiac toxicity in patients and in mice

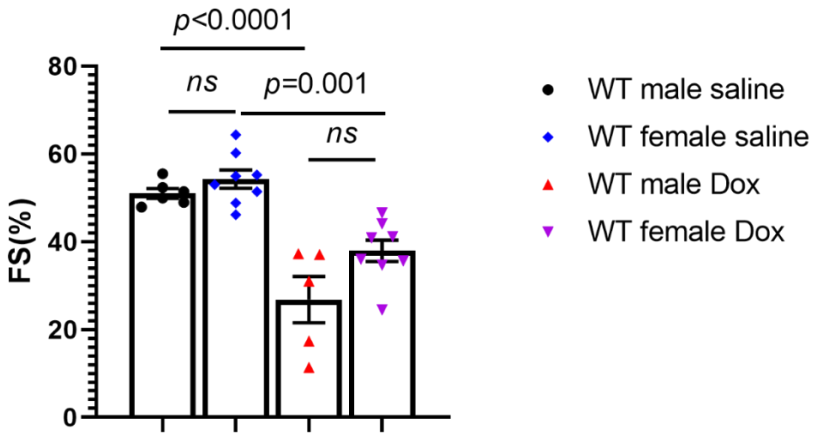
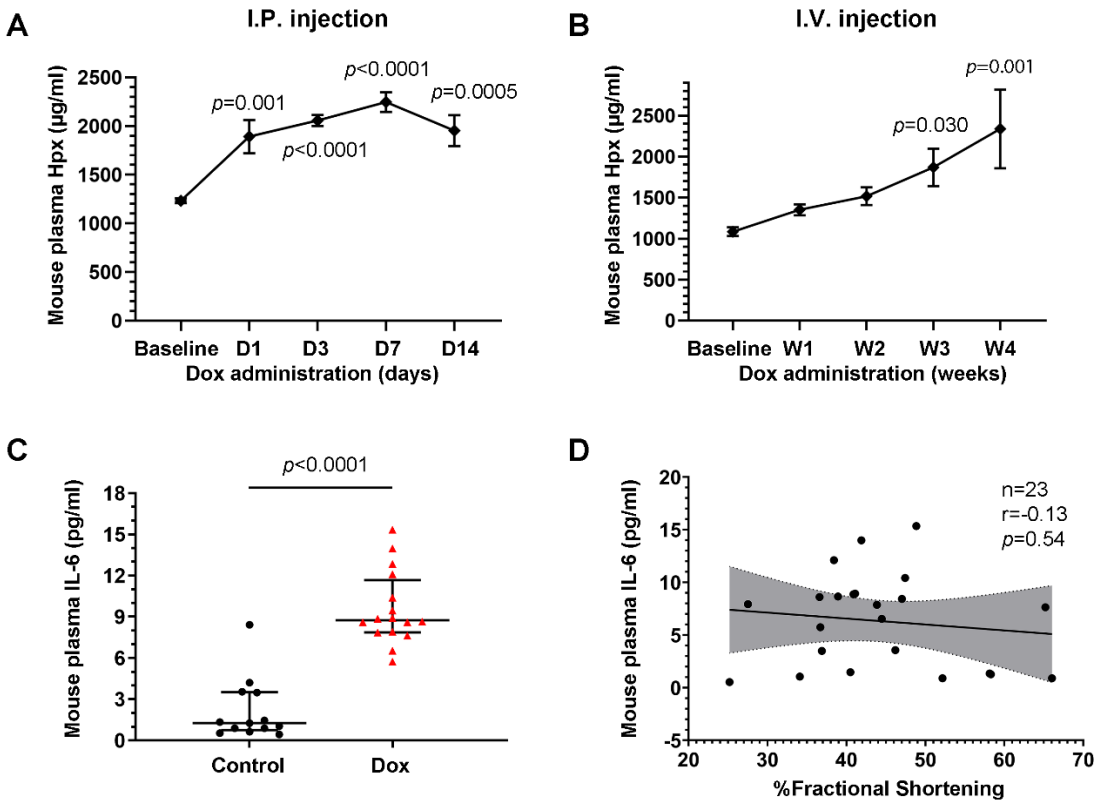


Fig. S1. Fractional shortening as assessed by echocardiography in male and female mice treated with saline or Dox.



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Protein	Pearson's R 3 months (n=30)	Nominal p value	Pearson's R 6 months (n=12)	Nominal p value
C-reactive protein	-0.10	0.6	0.11	0.7
Serum amyloid A	0.13	0.5	0.00	1.0
Alpha-1-antitrysin	0.08	0.7	-0.16	0.6
Haptoglobin	0.20	0.3	0.28	0.4

Fig. S2. (A). Mouse plasma Hpx levels in mice treated intraperitoneal (i.p) with Dox at baseline, day 1, 3, 7 and 14. n=7. (B). Mouse plasma Hpx levels in mice treated intravenous (i.v.) with Dox at baseline, week 1, 2, 3 and 4. n=7 for baseline, week 1, 2, 3 and n=5 for week 4. (C). Mouse plasma IL-6 levels in saline- (n=13) or Dox- (n=16) treated mice at 5 weeks post-treatment. (D). Pearson's correlation between mouse plasma IL-6 and mouse %FS. (E). Other proteins representing the acute phase inflammatory response were not associated with change in GLS at 3 or 6 months post-anthracyclines in the human discovery cohort. Data to support aptamer specificity was available for all proteins, as described in Table 2.

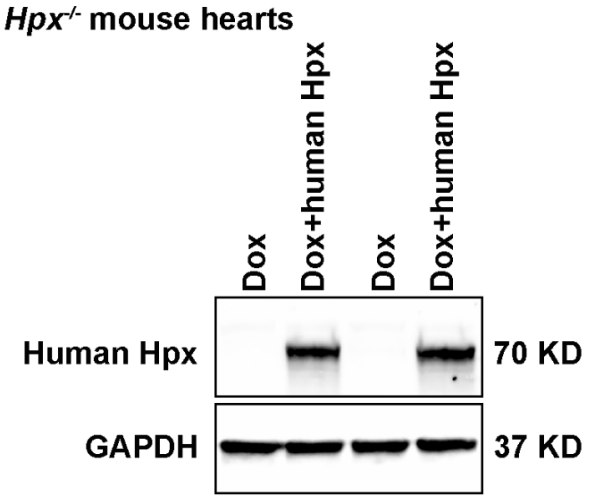

Fig. S3. Human Hpx levels in the hearts of *Hpx*-/- mice treated with Dox and purified human Hpx protein, as measured by Western Blot.

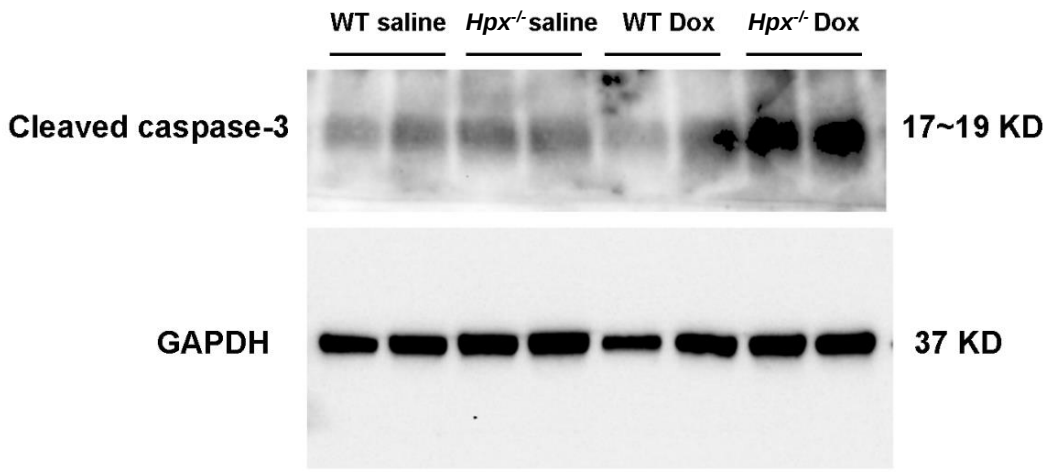

Fig. S4. Cleaved caspase-3 expression in the heart as measured by western blot.

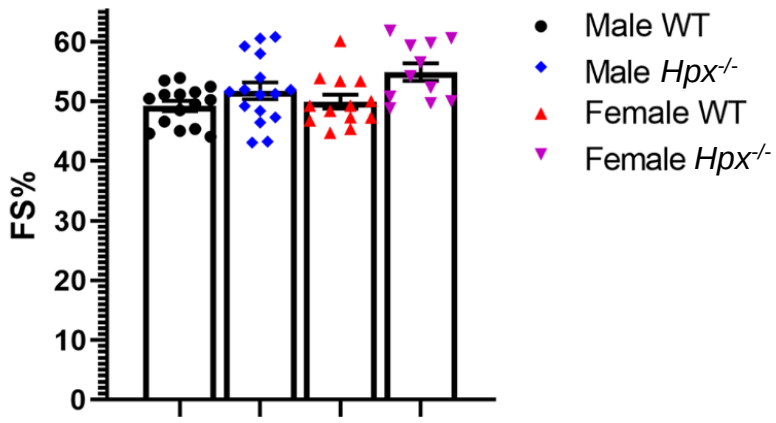

Fig. S5. Baseline cardiac function of WT and *Hpx*$^{-/-}$ mice before Dox treatment, as measured by fractional shortening (FS) on echocardiography.

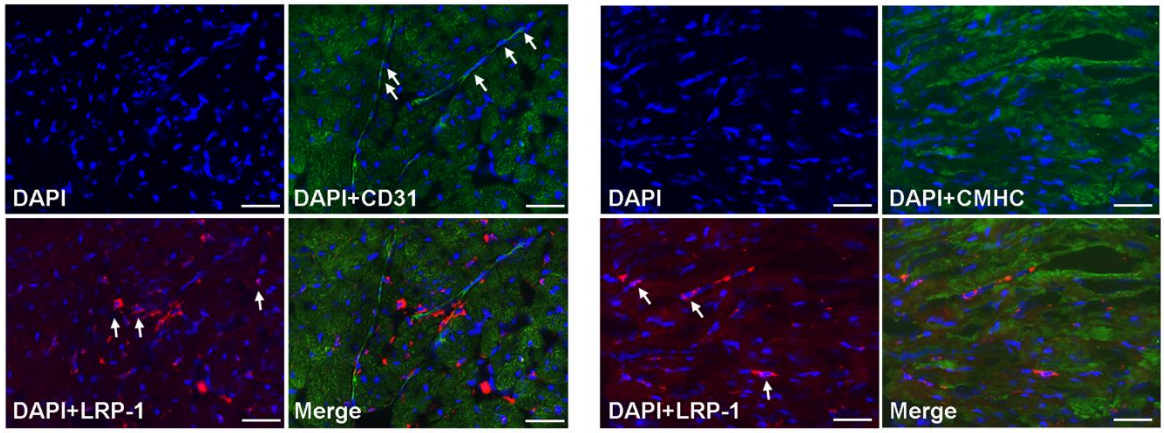

Fig. S6. The Hpx receptor LRP-1 does not co-localize with the endothelial cell marker CD31 or the cardiomyocyte marker cardiac myosin heavy chain (CMHC) in hearts isolated from WT mice treated with Dox. Arrows point to cells that were considered to be positive for CD31 or LRP-1, respectively. Scale: 50 μm.

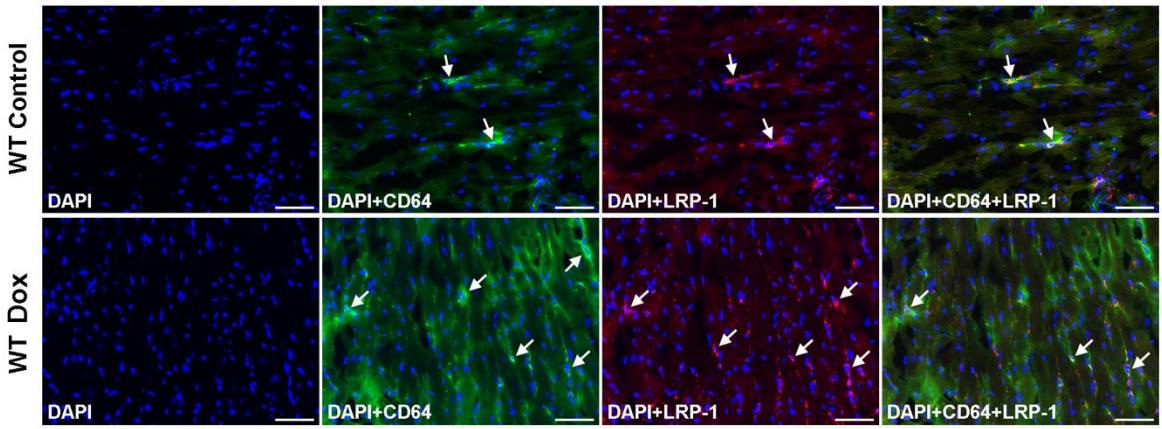

Fig. S7. The Hpx receptor LRP-1 co-localizes with $CD64^{+}$ immune cells in heart isolated from WT mice treated with saline or Dox. Arrows point to cells that were considered to be positive for CD64, LRP-1, or both, respectively. Scale: 50 μm.

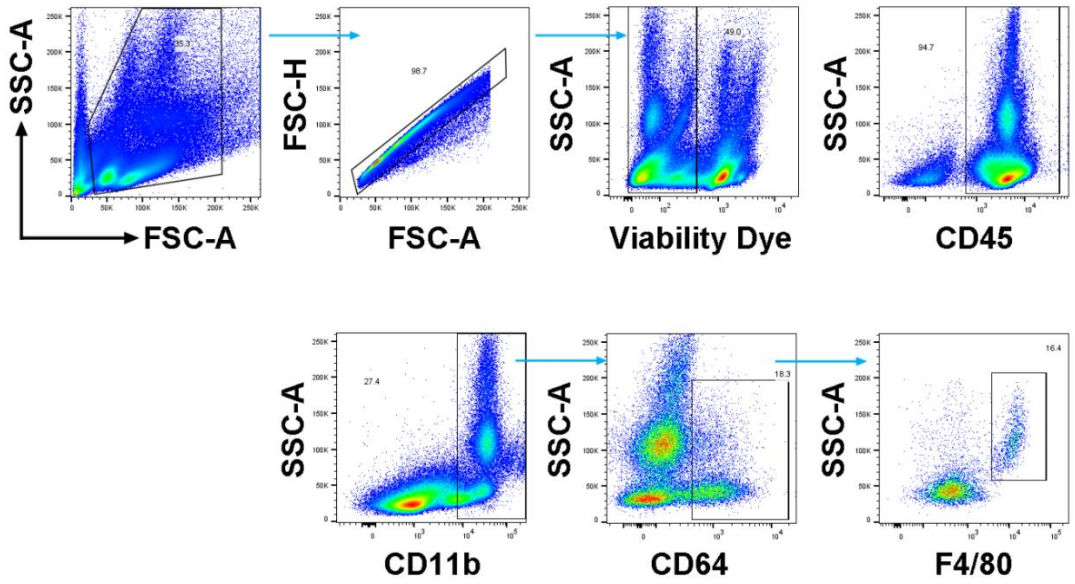

Fig. S8. Blood macrophage/monocyte isolation strategy.

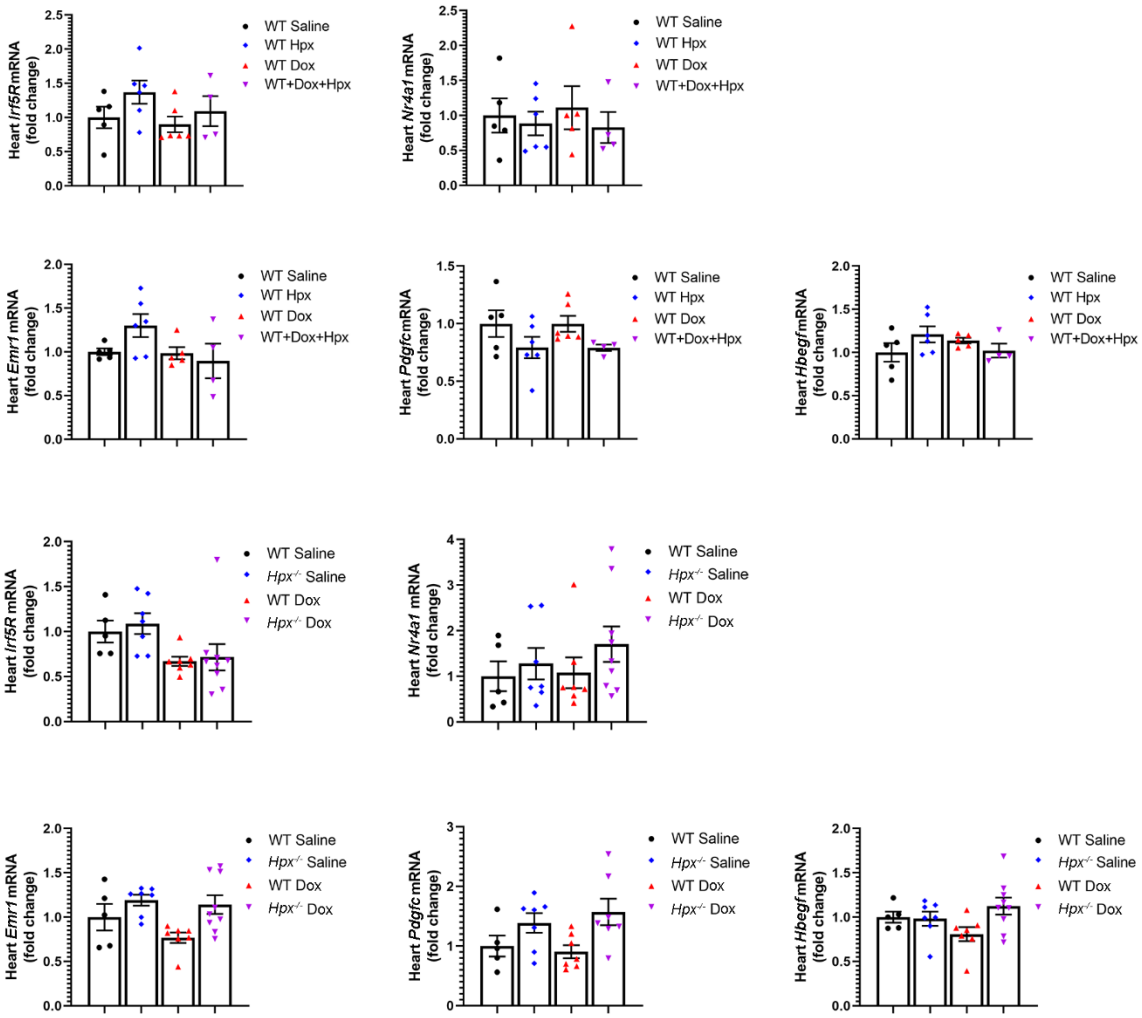

Fig. S9. Relative mRNA expression of genes representing inflammatory versus reparative macrophage phenotypes in the hearts of mice treated as indicated above.

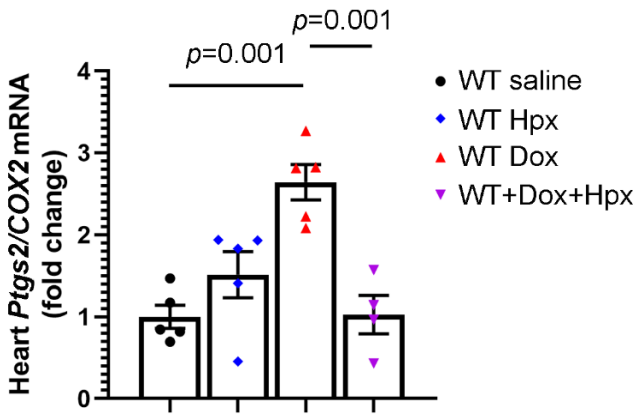

Fig. S10. *Ptgs2/COX2* expression in the hearts of mice treated as indicated above.

Supplemental Data: The Excel file “Supplemental data.xlsx” contains the following raw data from the two patient cohort studies: 1) SomaScan raw data from the Discovery Cohort; 2) Change in global longitudinal strain (GLS) versus change in plasma metabolite levels as measured previously by liquid chromatography-mass spectrometry in the Discovery Cohort; 3) GLS and plasma hemopexin levels (measured by ELISA) in the Validation cohort annotated by cancer type.